Deliverable D-JRP-TOXOSOURCES-WP3.4

Report on literature review on the prevalence of *T. gondii* oocysts in fresh produce and environment

Work package 3 of JRP22-FBZ4.1-TOXOSOURCES

Responsible Partners:
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D-JRP-TOXOSOURCES-WP3.4
REPORT ON LITERATURE REVIEW ON THE PREVALENCE OF *T. GONDII* OOCYSTS IN FRESH PRODUCE AND ENVIRONMENT

BACKGROUND

This is a public deliverable of One Health EJP Joint Research Project:
JRP22-FBZ4.1-TOXOSOURCES – *Toxoplasma gondii* sources quantified
(https://onehealthejp.eu/jrp-toxosources/);

Work Package:
JRP-TOXOSOURCES-WP3 Multicentre survey to fill the key existing gap: role of fresh produce (i.e. Ready-to-Eat salads);

Task:
JRP-TOXOSOURCES-WP3-T2 Design of a risk-based sampling strategy

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TOXOSOURCES addresses the research question – **What are the relative contributions of the different sources of *T. gondii* infection?** – by using several multidisciplinary approaches and novel and improved methods to yield robust estimates that can inform risk management and policy makers.

TOXOSOURCES WP3 aims to fill the knowledge gap concerning the relevance of fresh produce contamination by *T. gondii* oocysts as an infection source for humans.

Objectives of TOXOSOURCES WP3:

- ✓ To identify and assess the most appropriate procedure to detect *T. gondii* oocysts in fresh produce.
- ✓ To provide an overview of *T. gondii* oocysts in fresh produce and the environment.
- ✓ To conduct a risk-based pilot study based on available prevalence data (literature review), data on food production chains, EU trade patterns of selected fresh produce and available consumption data (WP2).
- ✓ To evaluate *T. gondii* oocyst contamination in selected fresh produce commodities by a multicentre pilot survey in representative EU regions.

As part of the work done in TOXOSOURCES WP3, existing data on the prevalence of *T. gondii* oocysts in fresh produce, as well as in the environment (soil and water), and relevant foods (i.e. bivalve mollusks) was reviewed. All related Milestones were reached on time.

This deliverable summarizes key aspects of the work. Results from the grey literature search are reported in this deliverable, while detailed description of methods and results of the systematic review conducted are reported in López-Ureña et al. (in preparation).

PURPOSE

The purpose of this work was to provide the most comprehensive systematic review on fresh produce and environmental contamination by *T. gondii* oocysts worldwide, to identify knowledge gaps related to sampling strategies and detection methods, and to formulate recommendations for harmonizing future studies.

METHODS

Three databases (PubMed, Web of Science and Scopus) and a different combination of search terms were used for systematic review following the PRISMA guidelines. Articles published up until December 2020, with no restriction on language, that reported direct detection of *T. gondii* in soil, water, fresh produce (vegetables and fruits) and/or bivalve mollusks were screened and selected, and their reference sections were examined to identify additional studies.

We included studies reporting direct detection of *T. gondii* oocysts in one of the matrices of interest with full text available. We excluded methodological studies aiming only to develop or improve oocyst recovery or detection methods (this part was extensively reviewed and reported in Deliverable D-JRP-TOXOSOURCES-WP3.1 and Slana et al., 2021), studies only involving experimental contamination, studies performed on other matrices, studies without available full text, and duplicates.

Three investigators carried out the initial screening focusing on title and abstract, and based on this, eligible articles were preselected and subjected to an in-depth review. Subsequently, data extraction was carried out by two co-authors and a third co-author resolved discrepancies. Data extraction and analysis are detailed in Lopez Urena et al. (in preparation). To quantify the heterogeneity within and among articles, the inverse variance index (I^2) was determined using STATA 12.0 software (StataCorp TX, USA).

Grey literature was also searched to obtain additional data on contamination of fresh produce and environment with *T. gondii* oocysts. An online questionnaire (administered using the Onlinesurveys platform) (Annex 1) was designed and sent out to all TOXOSOURCES consortium members and other collaborators together with an invitation letter (Annex 2).

KEY RESULTS AND DISCUSSION

Systematic review

Toxoplasma gondii *oocyst detection in environmental matrices*

Toxoplasma gondii oocysts were detected in all matrices worldwide based on molecular and/or bioassay methods, however there were many countries with no available information. In general, detection rates varied substantially by country and continent for the different types of matrices. Most of the studies were performed

in the Americas, where oocyst-associated toxoplasmosis outbreaks have been more frequently reported (Dubey, 2021) and where potentially more virulent strains of *T. gondii* circulate (Shapiro et al., 2019).

The first studies were performed in soil, right after the full life cycle of *T. gondii* was described (Dubey, 2009), followed by studies in water, bivalve mollusks and fresh produce, mirroring the increase of oocyst-related human toxoplasmosis outbreaks reported since 2000 (Pinto-Ferreira et al., 2019). Water was the matrix most frequenly studied, likely because human outbreaks associated with water contamination are the most frequently reported (Dubey, 2021).

Sampling strategies, detection methodologies and reporting

The studies selected were not comparable due to different sampling and methodological approaches. Thus, it is clear that harmonized procedures should be implemented in future studies.

Reporting of oocysts presence/quantification was also variable (i.e. number of oocysts per gram, or per sample, or per mL, or DNA volume or tachyzoite equivalent copies) preventing meaningful comparison of reported data. Level of contamination was up to 2,275 oocysts / mL in soil (da Silva & Langoni, 2016), 27,640 oocysts / sample in fresh produce (Lass et al., 2019) and 77,500 oocysts / sample in bivalves (Ghozzi et al., 2017).

Different methods were used for the recovery or concentration of *T. gondii* oocysts in the different matrices. They included washing, sedimentation, flotation, filtration or a combination of them, while some studies directly analyzed the samples. Higher variability among different matrices was observed regarding oocyst recovery compared with oocyst detection methods. In fresh produce and bivalve mollusks, a first key point for oocyst recovery was the sampling of individual *vs.* pooled samples. For the DNA extraction most of the studies included different freeze/thaw cycles or beat beating procedures, which may increase analytical sensitivity (Slana et al., 2021; Deliverable D-JRP-TOXOSOURCES-WP3.1). The detection of oocysts was mainly based on molecular methods. Genotyping tools were applied in few studies (Shapiro et al., 2015), despite they could be useful to trace outbreaks. Oocyst viability was confirmed in eleven studies (Ruiz et al. 1973; Ito et al. 1975; Coutinho et al. 1982; Dubey et al. 1995; Frenkel et al. 1995; Santos et al. 2010; Felicio et al. 2011; El-Tras et al. 2012; Minuzzi et al. 2021; Sroka et al. 2010; Vieira et al. 2015).

Grey literature

The search for grey literature on environmental contamination with *T. gondii* oocysts yielded seven reports with relevant information which are not findable as published in English-language peer-reviewed journals (Annex 3). These included theses, government reports and unpublished data, from Italy, Poland, Portugal, United Kindgom and Norway. Three reports focused on one matrix, whereas four reported on data from more than one matrix.

The single report investigating soil contamination in Poland did not find evidence of *T. gondii* oocyst contamination in farm gardens. Detection rates in water samples ranged from 0% in one study in Poland (N=24) assessing water recreation facilities and one in Italy (N=160) to 23.1% (N=65) in a study in Poland where water was sampled from household wells and water supply systems. Oocyst contamination of vegetables ranged from 0% of bulk vegetable (N=50) and ready-to-eat salad (N=25) samples in Portugal to 15.3% of samples (N=unknown) in one report from Italy. The only reports on bivalve mollusks were from Italy and the percentage of positive samples was in the order of 1.4% (N=2466) to 2.8% (N=871).

Differences in sampling strategies and in detection methods make it difficult to make comparisons between studies reported in the grey literature and also with published studies. What is particularly challenging for the grey literature is that in some cases there was very limited information on the sampling strategies and methodological approaches taken. Nevertheless in most reports reviewed here the detection methods employed were PCR or qPCR, accompanied by microscopy in some instances.

CONCLUSIONS

The worldwide detection rates reported for all environmental matrices, along with the published reports of confirmed human toxoplasmosis outbreaks due to contaminated water and fresh produce, evidence that environmental contamination with *T. gondii* oocysts poses a risk to public health. Both peer-reviewed and grey literature contribute to the current knowledge.

The overall detection rates of *T. gondii* were highly variable for each matrix, which can be partially explained by the different sampling strategies and methodologies employed. Therefore, guidelines for sampling strategy, oocyst recovery and detection for each matrix as well as reporting are needed in order to obtain robust and comparable results.

Through implementation of harmonized approaches in studies in the future, it will be possible to better assess the contribution of different environmental matrices as sources of *T. gondii* infection to humans and animals and provide appropriate advice to policy makers, food-producers and consumers. Oocyst viability determination and genetic characterization would be of major interest to definitively determine and characterize the risk and trace the sources.

DISSEMINATION AND IMPACT

This work provided relevant information to other work done in TOXOSOURCES WP2 and WP3. This information was used to define a sampling plan for a multi-center study investigating *T. gondii* contamination of RTE-salads at European level (WP3-T3) and the data were also delivered to TOXOSOURCES WP2 as input into the development of consumption survey and QMRA

Selected parts of this work or its results were presented as oral presentations at the ApicoWplexa meeting: Aplicomplexans and One Health (June 24th, 2021) and at the 13th European Multicolloquium of Parasitology (October 12th-16th, 2021) by Nadia María López-Ureña. This work and the results are part of a scientific manuscript that will be submitted to an Open Access journal. The scientific article will also form part of Nadia María López-Ureña's doctoral thesis at the Faculty of Veterinary at the University Complutense of Madrid.

This work was done in international multidisciplinary collaboration, and included both early-career and experienced scientists, and had thereby training, capacity-building and integrative aspects. The observations, approaches and results can shape future studies and improve their design and reporting, ultimately supporting evidence-based decisions and improving health.

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ANNEX 1. Questionnaire to gather data from grey literature on environmental contamination with *Toxoplasma gondii* oocysts

Question #	Question
1	What is the source of the information provided here? Please provide a complete reference, doi or weblink is availble.*
2	What samples type(s) were collected and analysed in the study?* • Vegetables • Fruit • Ready-to-eat salad • Mussels • Water • Soil • No data • Other (a) How many samples were collected in total?* (b) If you selected Other, please specify the sample type(s): i. How many samples of this type were collected? ii. Where were these samples collected? (c) How many vegetable samples were collected? (d) Please specify the types of vegetable collected and the number of samples of each type. Please write "no data" if this information is not provided. (e) How were the vegetables produced? • Conventional production • Organic production • No data • Other i. Please provide any further details here. (f) How were the vegetable samples presented? • Bulk • Packaged • Ready-to-eat • No data • Other i. Please provide any further details here: (g) Where were the vegetable samples collected? • Supermarkets • Markets • Farms • Storage • No data • Other i. If you selected Other, please specify: (h) How many fruit samples were collected? (i) Please specify the type(s) of fruit collected and the number of samples of each type. Please write 'no data' if this information is not provided. (j) How was the fruit produced? • Conventional production • Organic production • No data • Other i. Please provide any further details here. (k) How were the fruit samples presented? • Bulk • Packaged • Ready-to-eat

	• No data • Other i. Please provide any further details here. (l) Where were the fruit samples collected? • Supermarkets • Markets • Farms • Storage • No data • Other i. If you selected Other, please specify: (m) How may ready-to-eat salad samples were collected? (n) Please specify the type(s) of ready-to-eat salad collected and numbers of samples of each type. Please write 'no data' if this information is not provided. (o) How was the ready-to-eat salad produced? • Conventional production • Organic production • No data • Other i. Please provide any further details here. (p) Where were the ready-to-eat salada samples collected? • Supermarkets • Markets • Farms • Storage • No data • Other i. If you selected Other, please specify: (q) How many mussel samples were collected? (r) Where were the mussel samples collected? Please write "no data" if this information is not provided. (s) How many water samples were collected? (t) Please specify the source of the water samples and the number of samples collected from each source. Please write "no data" if this information is not provided. (u) How many soil samples were collected? (v) Please specify the type(s) of soil sampels collected. Please write "no data" of this information is not provided. (w) Where were the soil samples collected? Please write "no data" if this information is not provided.
3	In which year were the samples collected? Please write "no data" if this information is not provided.*
4	In which season were the samples collected?* • Spring • Summer • Autumn • Winter • No data
5	In which country were samples collected? Please write "no data" if this information is not provided.*
6	How much of each sample was collected (in grams, mL or units)? Please write "no Data" if this information is not provided.
7	How were oocysts detected?* • PCR • Quantitative PCR • Microscopy • No data • Other

	(a) If you selected Other, please specify:
8	Briefly describe the sample preparation method(s) used (e.g. information about the sample collection and preparation process, prior to oocyst recovery and detection). Please provide details for each matrix/sample type analysed. Please write "no data" if this information is not provided.
9	What oocyst recovery and concentration method(s) was/were used?* • Filtration • Flocculation • Wash • Sedimentation • Centrifugation • No data • Other (a) If you selected Other, please specify the method(s) used for each sample type: i. What was the source of this/these methods? • In-house • Official • No data • Other If you selected Other, please specify: (b) What was the source of the filtration method used for oocyst recovery? • In-house • Official • No data • Other i. If you selected Other, please specify: (c) From which sample type(s) were oocysts recovered using filtation? • Vegetables • Fruit • Ready-to-eat salad • Mussels • Water • Soil • No data • Other (d) What was the source of the flocculation method used for oocyst recovery? • In-house • Official • No data • Other i. If you selected Other, please specify: (e) From which sample types were oocysts recovered using flocculation? • Vegetables • Fruit • Ready-to-eat salad • Mussels • Water • Soil • No data • Other (f) What was the source of the wash method used for oocysts recovery? • In-house • Official • No data • Other i. If you selected Other, please specify: (g) From which sample types were oocysts recovered using the wash method? • Vegetables

	• Fruit • Ready-to-eat salad • Mussels • Water • Soil • No data • Other (h) What was the source of the sedimentation method used for oocyst recovery? • In-house • Official • No data • Other i. If you selected Other, please specify: (i) From which samples types were oocysts recovered using the sedimentation method? • Vegetables • Fruit • Ready-to-eat salad • Mussels • Water • Soil • No data • Other (j) What was the source of the centrifugation methods used for oocysts recovery? • In-house • Official • No data • Other i. If you selected Other, please specify: (k) From which sample types were oocysts recovered using the centrifugation method? • Vegetables • Fruit • Ready-to-eat salad • Mussels • Water • Soil • No data • Other
10	If molecular methods were employed, which DNA extraction method was used?* • Column purification • Phenol chloroform isoamyl alcohol • No data • Not applicable • Other (a) If you selected Other, please specify: (b) Please provide a reference for the DNA extraction method if available.
11	What molecular marker(s) was/were used for oocyst detection?* • B1 • 529 bp • 18S • No data • Not applicable • Other (a) If you selected Other, please specify:
12	Please provide a summary of the oocyst recovery, concentration and detection methods used. Please write "no data" if this information is not provided.

13	What was the total number of *Toxoplasma* positive samples? Please write "no data" if this information is not provided.
14	What was the percentage of *Toxoplasma* positive samples? Please write "no data" if this information is not provided.
15	Which sample types were positive for *Toxoplasma*? • Vegetables • Fruit • Ready-to-eat salad • Mussels • Water • Soil • No data • Other (a) If you selected Other, please specify: (b) How many vegetable samples were *Toxoplasma* positive? If several different vegetable products were positive, please specify type and number positive for each. Please write "no data" if this information is not provided. (c) What percentage of vegetable samples were *Toxoplasma* positive? If several different vegetable products were positive, please specify type and percentage positive for each. Please write "no data" if this information is not provided. (d) How many fruit samples were *Toxoplasma* positive? If several different fruit products were positive, please specify type and number positive for each. Please write "no data" if this information is not provided. (e) What percentage of fruit samples were *Toxoplasma* positive? If several different fruit products were positive, please specify type and percentage positive for each. Please write "no data" if this information is not provided. (f) How many ready-to-eat salad samples were *Toxoplasma* positive? If several different ready-to-eat salad products were positive, please specify type and number positive for each. Please write "no data" if this information is not provided. (g) What percentage of ready-to-eat samples were *Toxoplasma* positive? If several different ready-to-eat salad products were positive, please specify type and percentage positive for each. Please write "no data" if this information is not provided. (h) How many mussel samples were *Toxoplasma* positive? Please write "no data" if this information is not provided. (i) What percentage of vegetable samples were *Toxoplasma* positive? Please write "no data" if this information is not provided. (j) How many water samples were *Toxoplasma* positive? If samples from several different water sources were positive, please specify source and number positive for each. Please write "no data" if this information is not provided. (k) What percentage of water samples were *Toxoplasma* positive? If samples from several different water sources were positive, please specify source and percentage positive for each. Please write "no data" if this information is not provided. (l) How many soil samples were *Toxoplasma* positive? If several different soil types were positive, please specify type and number positive for each. Please write "no data" if this information is not provided. (m) What percentage of soil samples were Toxoplasma positive? If several different soil types were positive, please specify type and percentage positive for each. Please write "no data" if this information is not provided. (n) How many other samples were *Toxoplasma* positive? If several different other sample types were positive, please specify type and number positive for each. Please write "no data" if this information is not provided. (o) What percentage of other samples were *Toxoplasma* positive? If several different other sample types were positive, please specify type and percentage positive for each. Please write "no data" if this information is not provided.
16	What was the median and range number of oocysts detected per gram or mL or per sample? (Please include units). Please write "no data" if this information is not provided or "not applicable" if not relevant to this study.*

17	Does the study report the analytical sensitivity (lowest level of detection) of the oocyst detection method used?* • YES • NO (a) If YES, please specify (in oocysts per gram or mL sample).
18	Please list any other parasites investigated in the study. Please write "no data" if this information is not provided.*
19	Please provide a summary of the main findings of the study.*
20	Please eneter the name and email address of the person who completed the questionnaire. Please note that provision of this personal information is optional. We are requesting it so we can contact you if we require any further information.

* Questions for which an answer is required. All other questions are optional.

NB. The online questionnaire was designed in such a way that certain questions only appeared if the respondents inputted certain responses. Thus respondents did not generally see all the questions listed above.

ANNEX 2. Invitation letter

Dear TOXOSOURCES colleagues,

As part of the TOXOSOURCES WP3-T2, we are conducting a literature review on prevalence of Toxoplasma gondii (oocysts) (observational studies) in fresh produce (i.e. fruit and vegetables), bivalves and environmental (soil and water) samples in Europe to be implemented by collecting data from grey literature. We would be very grateful for your assistance in this task to access and collect data from unpublished scientific information, including reports from governmental agencies, thesis dissertations, conference proceedings, and other grey literature that might be only available locally or in a local language. You can help us by accessing any relevant information you can find on T. gondii oocyst contamination of fresh produce, bivalves and environmental samples in Europe and completing the questionnaire below.

https://surrey.onlinesurveys.ac.uk/toxosources-fresh-produce-and-environmental-contamination-5

The questionnaire can be completed multiple times and we ask you to complete a separate questionnaire for each study or report you have identified. Please mark any fields that you cannot complete as 'no data'.
Please do get in touch with us if you have any further questions. We would be very grateful if you could complete the questionnaire by 31st August 2020.

Many thanks for your participation in TOXOSOURCES WP3-T2.
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Martha Betson, UoS (m.betson@surrey.ac.uk), TOXOSOURCES WP3-T2 Deputy Task Leader
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ANNEX 3. Summary of the reviewed grey literature on environmental contamination with *T. gondii* oocysts

Matrix	Country	Sample number collected	Sample volume / mass	Sampling date	Additional sample information	Oocyst recovery method	Detection methods (molecular target if applicable)	Positive samples (%)	Information source
Soil	Poland	70	100 g	2001-2002 in spring & summer	Soil from backyard gardens of farms	Filtration, centrifugation, flotation	Microscopy, PCR (B1)	0 (0) by microscopy and PCR	"Studies on *Toxoplasma gondii* occurrence in farm and wild animals from area of Lublin province as a threat to rural inhabitants health" doctoral dissertation, Jacek Sroka (2005), Faculty of Veterinary Medicine at the Agricultural University in Lublin
Water	Poland	65	5 L	2001-2002 in spring & summer	Household wells (53), water supply systems (12)	Filtration, centrifugation, flotation	Microscopy, PCR (B1)	12 (18.5) by microscopy 15 (23.1) by PCR	"Studies on *Toxoplasma gondii* occurrence in farm and wild animals from area of Lublin province as a threat to rural inhabitants health" doctoral dissertation, Jacek Sroka (2005), Faculty of Veterinary Medicine at the Agricultural University in Lublin
Water	Poland (Krakow)	24	10 L	2014 in summer & autumn	Open (4) and closed (4) water recreation facilities. Samples collected three times from each water source	Filtration, wash, centrifugation and flotation	PCR (B1)	0 (0)	doi: 10.15199/62.2016.1.18
Water	Italy (north-east)	160	No data	2000-2010	None	Centrifugation	Microscopy, PCR (18S rRNA)	0 (0)	Report 2000-2020 IZS Lombardia and Emilia Romagna (data from laboratory database)
Raw water	Scotland, UK	179	No data	Feb-June 2013	Samples collected from 68 water plants throughout Scotland	Filtration, centrifugation	qPCR (529 bp)	1 (0.56)	"*Toxoplasma gondii* in animals and the Environment" Doctorate of Research in Veterinary Science thesis, Maria Parigi (2014), Università di Bologna
Drinking water	Scotland, UK	179	No data		Samples collected from 68 water	Filtration, centrifugation	qPCR (529 bp)	0 (0)	"*Toxoplasma gondii* in animals and the Environment" Doctorate of

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					plants throughout Scotland				Research in Veterinary Science thesis, Maria Parigi (2014), Università di Bologna
Vegetables	Italy	137	No data	2017-2018	None	No data	No data	18 (13.1) - 2018 15.3% - 2017	https://www.salute.gov.it/imgs/C_17_pubblicazioni_2938_allegato.pdf
Fruit	Italy	2	No data	2017-2018	None	No data	No data	0 (0) – 2018	https://www.salute.gov.it/imgs/C_17_pubblicazioni_2938_allegato.pdf Report 2000-2020 IZS Lombardia and Emilia Romagna (data from laboratory database)
Vegetables	Italy (north-east)	476	5 g	2000-2010	Various types including salad, arugula, spinach. Bulked, packaged & ready-to-eat	Wash and centrifugation	Microscopy, PCR (18S rRNA)	20 (4.2)	
Fruit	Italy (north-east)	118	No data	2000-2010	None	Wash and centrifugation	Microscopy, PCR (18S rRNA)	0 (0)	Report 2000-2020 IZS Lombardia and Emilia Romagna (data from laboratory database) "Coating of immunomagnetic beads with a monoclonal antibody for separation and concentration of *Toxoplasma gondii* oocysts" Master's thesis Universidade nova de Lisboa; "Microbiological and parasitological study comparing unwashed and ready to eat salads" Master's thesis Universidade nova do Porto
Ready-to-eat salad	Italy (north-east)	360	No data	2000-2010	Mixed salad	Wash and centrifugation	Microscopy, PCR (18S rRNA)	6 (1.7%)	
Vegetables	Portugal (Lisbon area)	50	25 g	2006 in autumn & winter	Lettuce, carrots, tomatoes. Conventional production. Packaged, ready-to-eat. Obtained from supermarkets	Wash	PCR (B1)	0 (0)	
Ready-to-eat salad	Portugal (Lisbon area)	25	25 g	2006 in autumn & winter	Conventional production. Obtained from supermarkets and markets.	Wash	PCR (B1)	0 (0)	"Coating of immunomagnetic beads with a monoclonal antibody for separation and concentration of *Toxoplasma gondii* oocysts" Master's thesis Universidade nova de Lisboa; "Microbiological and parasitological study comparing unwashed and ready to eat salads" Master's thesis Universidade nova do Porto Unpublished data from ParaBerry project, details not shown
Fruit - blueberries	Norway								
Fruit – raspberries	Norway								Unpublished data from ParaBerry project, details not shown

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Fruit - strawberries	Norway								Unpublished data from ParaBerry project, details not shown
Bivalve mollusks	Italy	871	No data	2017-2018	None	No data	No data	24 (2.8) – 2018 2.5% - 2017	https://www.salute.gov.it/imgs/C_17_pubblicazioni_2938_allegato.pdf
Bivalve mollusks	Italy (north-east)	2466	25-75 mg tissue (no haemolymph)	2000-2010	Mussels (*Mytilus galloprovincialis*, oysters (*Crassostrea gigas*); clams (*Venus gallina, Tapes semidecussa, Tapes philippinarum*)	Apparently direct	Microscopy, PCR (18S rRNA)	35 (1.4)	ort 2000-2020 IZS Lombardia and Emilia Romagna (data from laboratory database)