7th Proficiency Test on "Molecular identification of Anisakid nematodes at the species level" – PT07

European Union Reference Laboratory for Parasites
Istituto Superiore di Sanità

XVIII Workshop of National Reference Laboratories for Parasites
Rome, 16th-17th November 2023

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Aim of the Proficiency Test

Evaluating the performance of the participant laboratory at correctly identifying larvae of anisakid nematodes at the species level applying molecular methods.

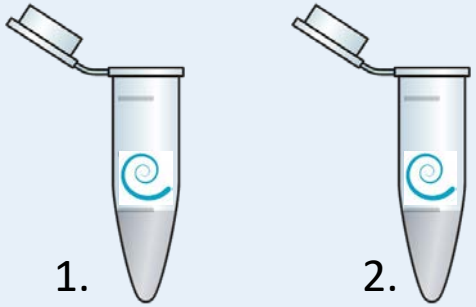
The PT is accredited according to the ISO 17043.

Timeline

Call for participing	10th January
Deadline for subscription	24th February
Pt sending	13th March
Deadline for submitting results	14th April
Individual report	24th April
Final report published on the EURLP webpage	22th May

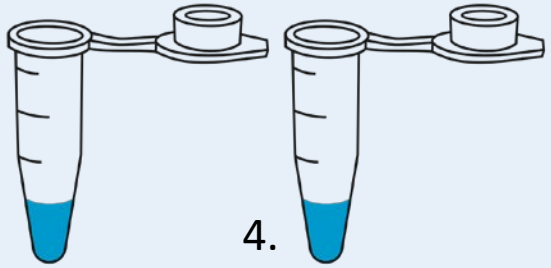
PT panel

The single PT panel consisted of **4** items:



Pseudoterranova decipiens

Anisakis pregreffii

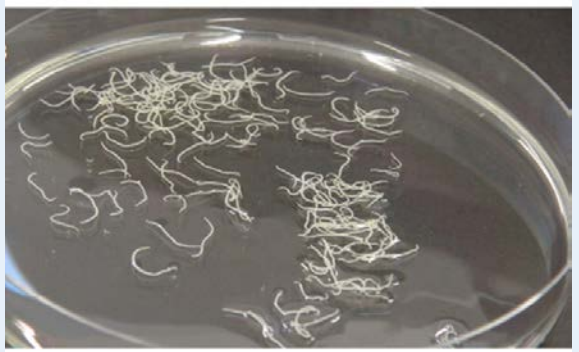


Anisakis simplex ss

Contracoecum rudolphii C

L3 larva fragment

Extracted DNA

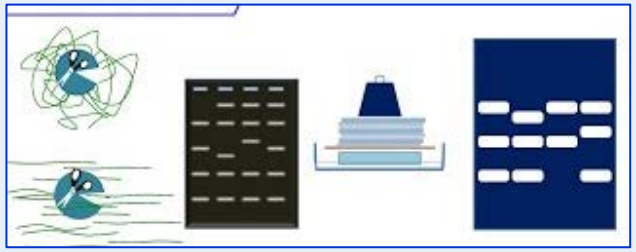


All larvae have been previously identified at species level by analyzing one of their fragments by the EURLP method “Identification at species level of parasites of the family *Anisakidae* by PCR/RFLP”, MI04, same for DNA.

Molecular identification

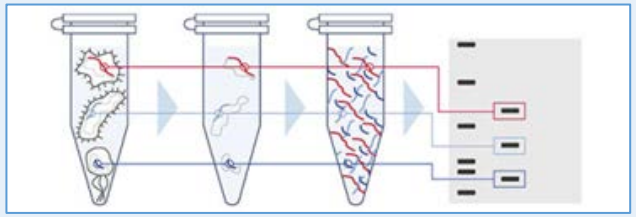
Recommended methods:

A) "Identification at species level of parasites of the family Anisakidae by **PCR/RFLP**" MI04



A. simplex ss.
A. pegreffi
A. simplex/pegreffi hybrid
A. simplex C
A. ziphidarium
A. physeteris
A. typica
A. sp. A
Pseudoterranova spp
Hysterotilacium spp
Contracaecum rudolphii (A, B, C)

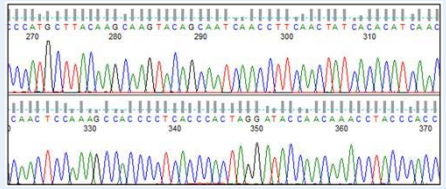
B) "Identification of Anisakidae Larvae at the species level by **multiplex** PCR" MI10



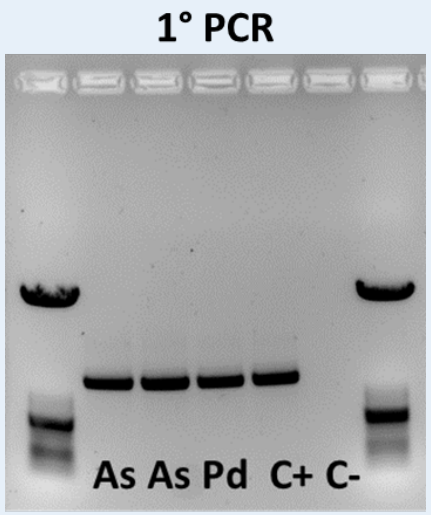
A. pegreffii
A. simplex s.l. (incl. *A. simplex/pegreffii hybrid*)
A. physeteris (incl. *A. brevispiculata* and *A. paggiae*)
A. typica
Pseudoterranova spp
Hysterotilacium spp (H. aduncum)
Contracaecum rudolphii (A, B, C)

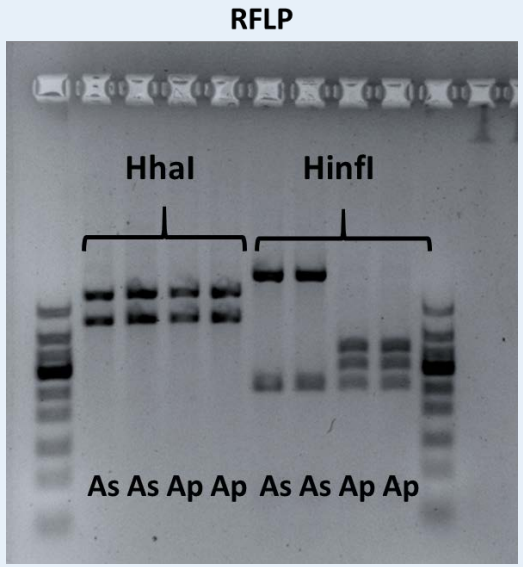
OR:

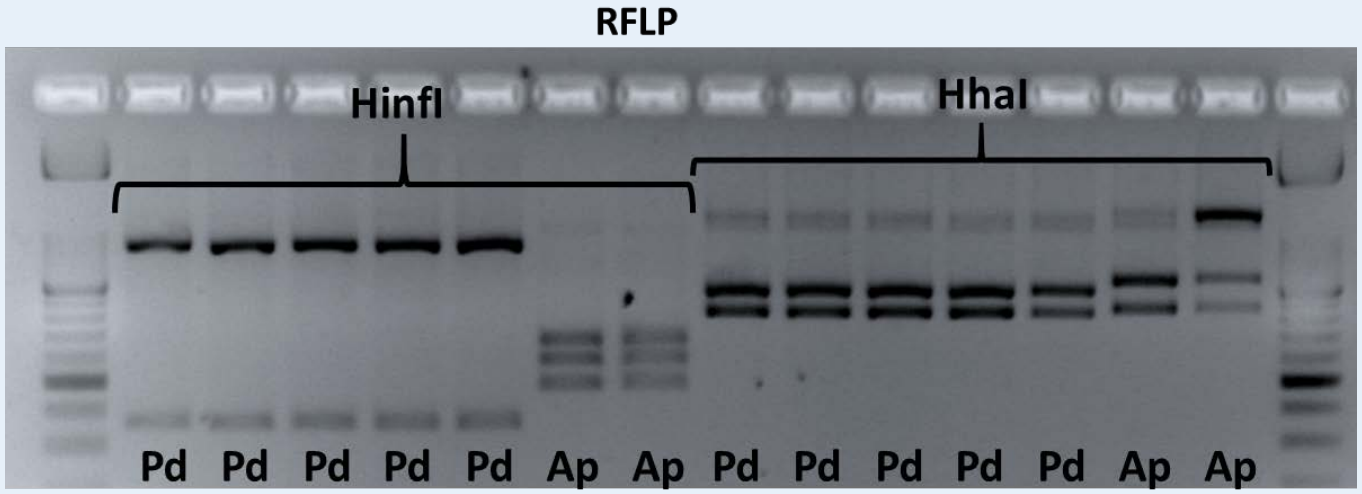
C) Any other suitable molecular method performed by the participant laboratory (i.e. PCR and sequencing)

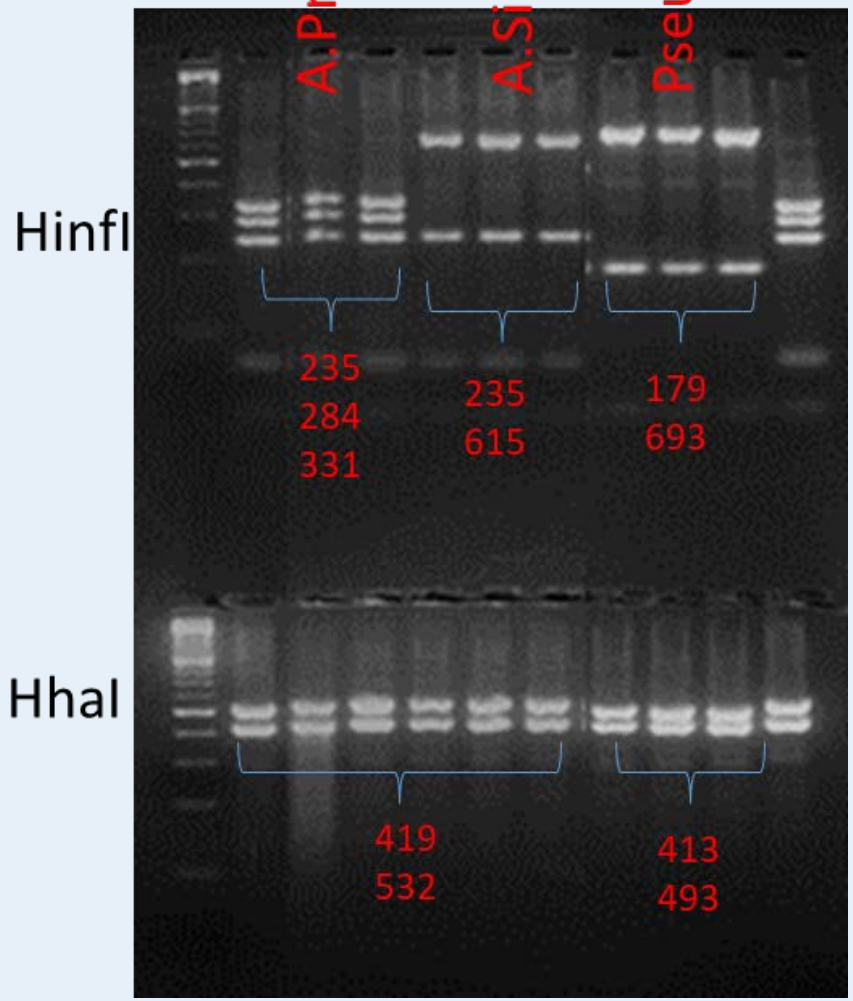


Identification at species level of parasites of the family *Anisakidae* by PCR/RFLP









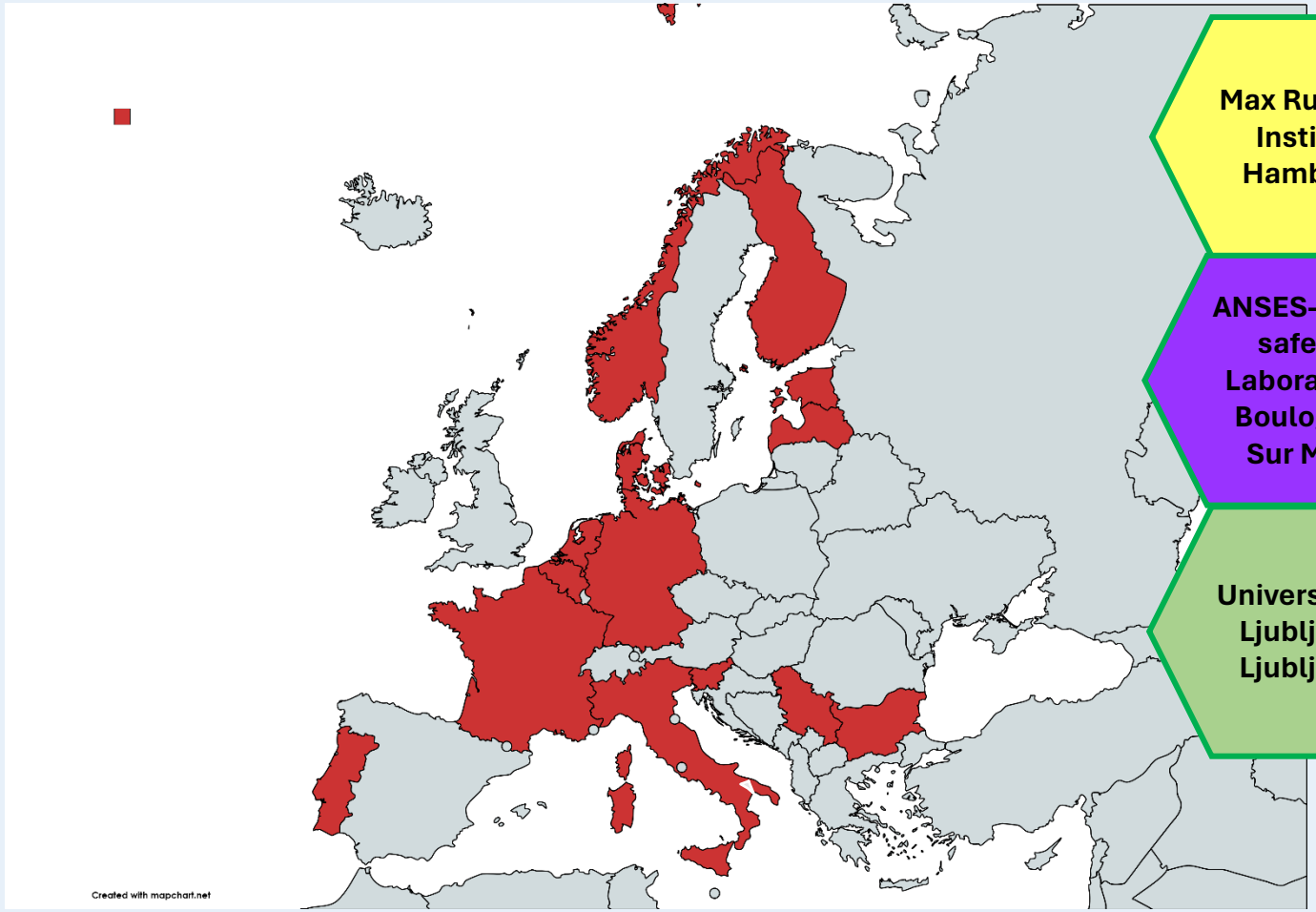
Evaluation criteria

The PT evaluation is only **qualitative** and no statistical analysis of the results is applied.

For each PT item the result is **"correct"** or **"incorrect"** according to the correct or incorrect identification

The PT is considered "positive" if no "incorrect" results were obtained; the PT is considered "negative" if at least one "incorrect" result was obtained

PT participants



- Statens Serum Institut Copenhagen
- Estonian Veterinary and Food Laboratory Tartu
- Max Rubner-Institut Hamburg
- National Reference Laboratory for Anisakiasis Palermo
- Institute for diagnosis and animal health, Bucharest
- Institute of Veterinary Medicine of Serbia, Belgrade
- ANSES-Food safety Laboratory Boulogne Sur Mer
- Institute of food safety, animal health and environment Riga
- Institute of Tropical Medicine Antwerp
- Finnish Food Safety Authority Evira, Oulu
- University of Ljubljana Ljubljana
- Institute for Public Health and Environment Bilthoven
- Institute of Marine Research Bergen
- Instituto nacional de investigacao agraria e veterinaria Lisbon

14 participating laboratories

Results-1

Participation

- **14/14** labs sent the results

Methods

- **5** PCR-RFLP
- **1** PCR-RFLP and sequencing
- **1** PCR-RFLP and Multiplex PCR
- **4** PCR Multiplex
- **3** PCR + Sequencing (COX1, COX2 or universal primers)

7 PCR-RFLP

5 PCR Multiplex

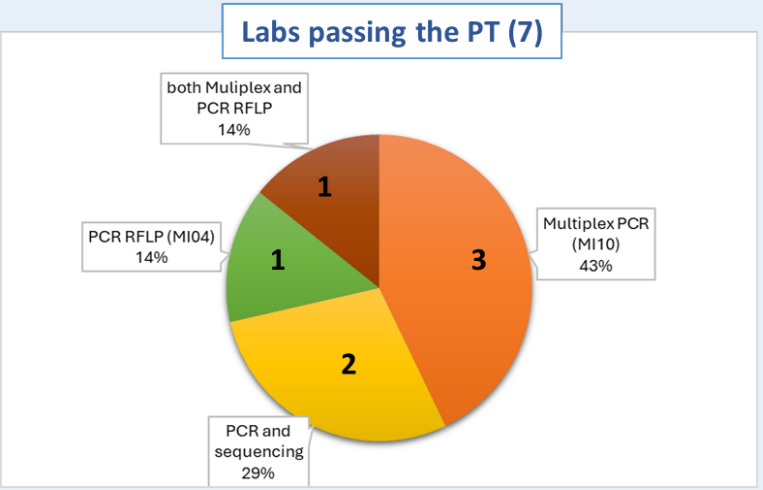
4 sequencing

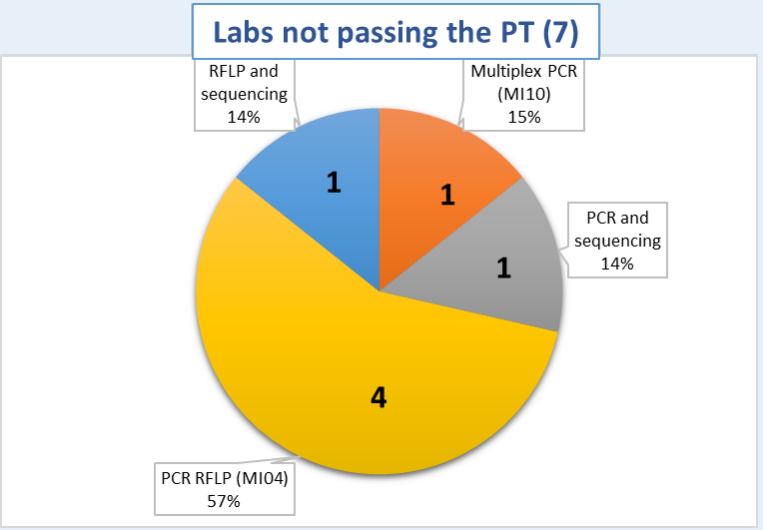
Results-2

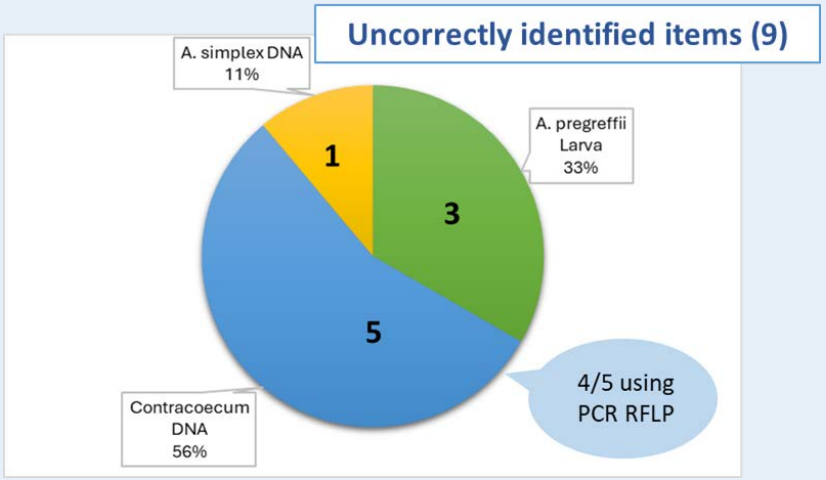
Detection

7/14 (**50%**) of the reporting laboratories passed the PT

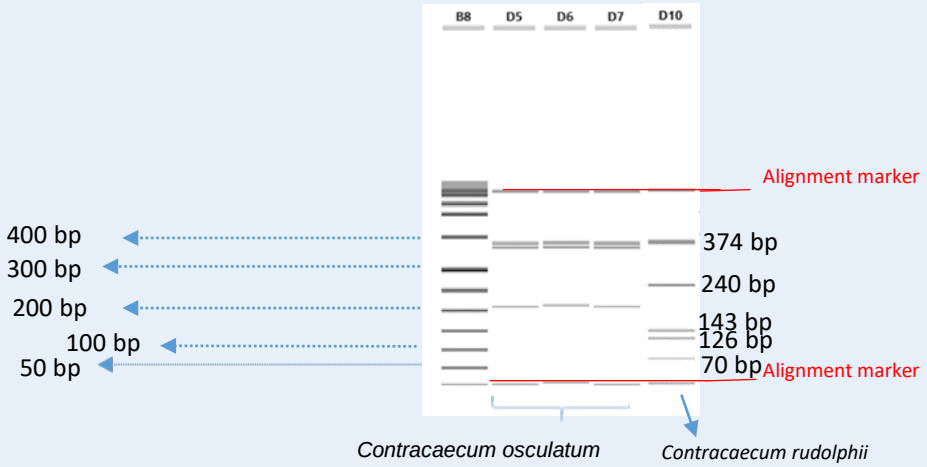
Laboratory code	N° of samples correctly identified		N° of samples NOT correctly identified		Method(s)	Final evaluation
	Larva	DNA	Larva	DNA		
A3	2	1	0	1	EURLP method 1 (PCR_RFLP);	**Negative**
A6	2	2	0	0	EURLP method 2 (multiplex PCR);	**Positive**
A7	2	2	0	0	EURLP method 1 (PCR_RFLP); EURLP method 2 (multiplex PCR);	**Positive**
A8	1	0	1	2	cox1 PCR and sequencing;	**Negative**
A10	2	2	0	0	EURLP method 1 (PCR_RFLP);	**Positive**
A12	2	1	0	1	EURLP method 1 (PCR_RFLP);	**Negative**
A15	1	2	1	0	EURLP method 1 (PCR_RFLP); PCR and sequencing	**Negative**
A16	2	2	0	0	EURLP method 2 (multiplex PCR);	**Positive**
A26	2	1	0	1	EURLP method 1 (PCR_RFLP);	**Negative**
A28	2	2	0	0	PCR (universal primers) and sequencing	**Positive**
A31	2	1	0	1	EURLP method 1 (PCR_RFLP);	**Negative**
A38	1	2	1	0	EURLP method 2 (multiplex PCR);	**Negative**
A39	2	2	0	0	cox2 PCR and sequencing;	**Positive**
A40	2	2	0	0	EURLP method 2 (multiplex PCR);	**Positive**





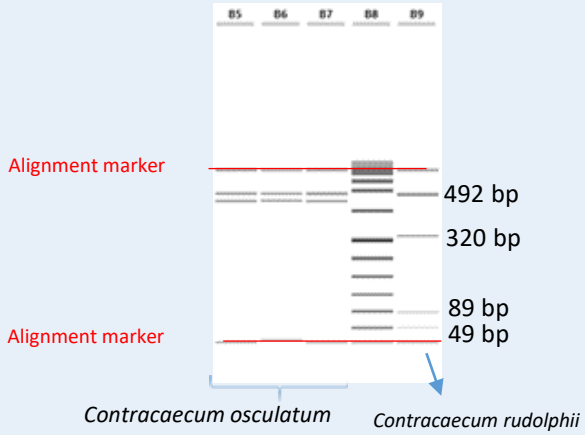


HhaI



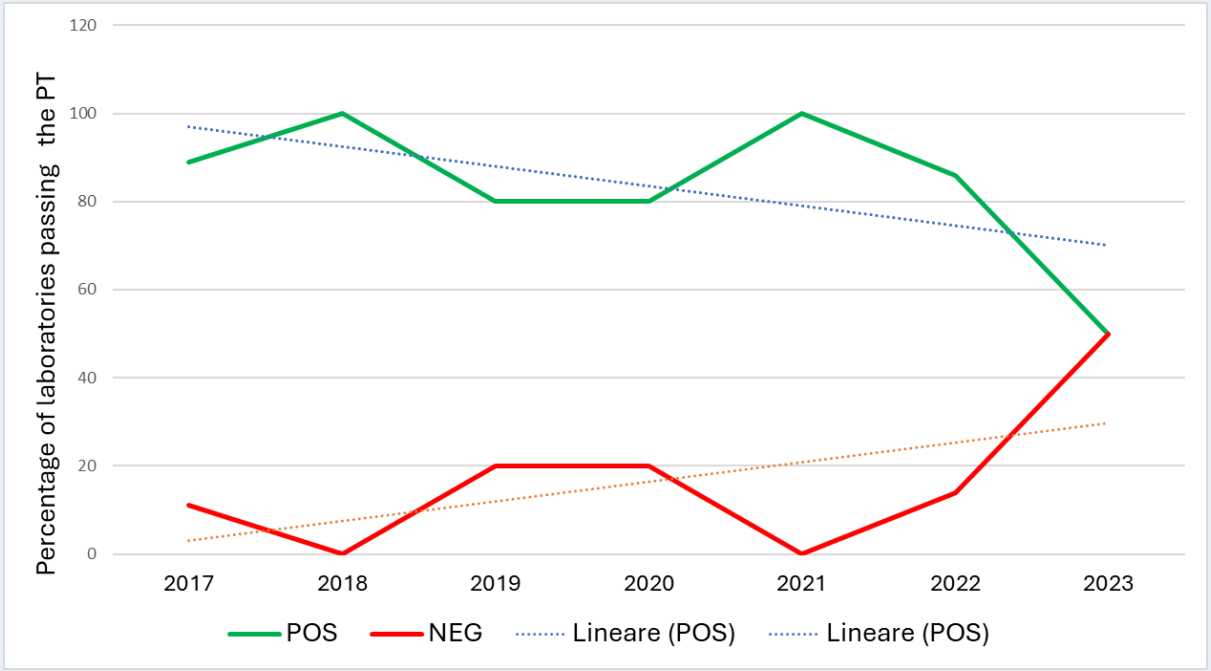
	PCR fragments of ITS amplicon	
Enzimi di restrizione	***HinfI***	***HhaI***
Contracaecum rudolphii (A, B, C)	**3, 49, 89, 320, 492**	**70, 126, 143, 240, 374**
Contracaecum osculatum	**463-501**	**212, 375, 377**

HinfI

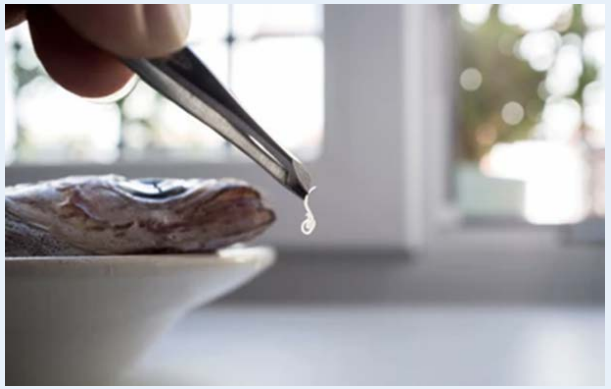


PT trend

Lab code	2017	2018	2019	2020	2021	2022	2023
A3							
A6							
A7							
A8							
A10							
A11		na					
A12							
A15							
A16							
A17							
A20							
A26					na		
A28							
A31		na					
A38							
A39							
A40				na			



Conclusions



- 7/14 reporting laboratories passed the PT.
- The PCR-RFLP and Multiplex PCR are the preferred methods.
- Negative trend in 2023.
- What seems positively correlated to a positive evaluation **is a long-time experience**, but also the method of choice. Method of choice may influence the performance when less common Anisakid species are in the panel.

Thanks

See you in 2024!

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