

# PT-05

## DETECTION OF *ECHINOCOCCUS* SPP. WORMS IN THE INTESTINAL MUCOSA OF THE DEFINITIVE HOST

Azzurra SANTORO, Francesco CELANI, Simona CHERCHI, Chiara CURRA', Federica  
SANTOLAMAZZA

**Workshop of the National Reference Laboratories for Parasites,  
Rome, Italy, 28-29 October 2025**



European Union Reference Laboratory  
for Parasites (EURL-P; food safety)



# THE PT IS ACCREDITED IN A QUALITY SYSTEM ACCORDING TO ISO 17043 STANDARD



**AIM:** to test the competence of the appointed NRLs to detect worms of *Echinococcus* sp. in a matrix made by intestinal mucosa.

**PT PANEL:** consists in three tubes filled with homogenized intestinal mucosa spiked or not with worms of *Echinococcus* spp.



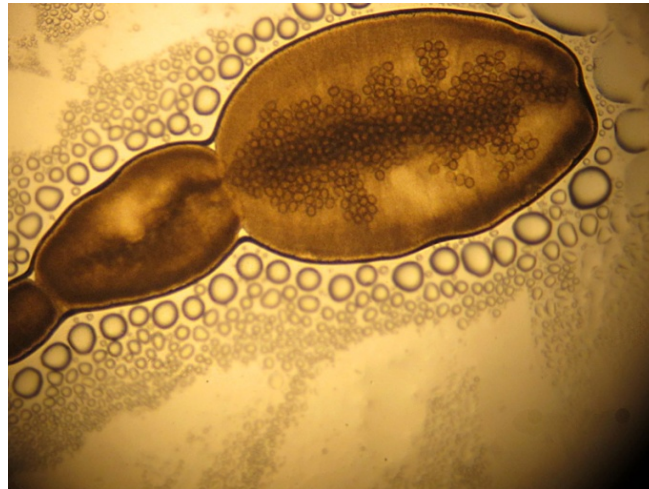
|                           |           |                                             |           |
|---------------------------|-----------|---------------------------------------------|-----------|
| CALL FOR PARTICIPING      | 02-3rd    | DEADLINE FOR SUBMITTING RESULTS             | 03 – 24th |
| DEADLINE FOR SUBSCRIPTION | 02 – 24th | INDIVIDUAL REPORT                           | 03 – 28th |
| PT SENDING                | 03 – 17th | FINAL REPORT PUBLISHED ON THE EURLP WEBPAGE | 03 – 28th |

# CRITERIA FOR THE QUALITATIVE EVALUATION

- Each PT item is CORRECT if participant detected one or more *Echinococcus* spp. worms in spiked samples OR no worms in not spiked samples...

...OR INCORRECT in case of false positive or false negative results.

- The FINAL EVALUATION is only based on qualitative evaluation and is expressed as “POSITIVE” if the results of all samples are correct OR “NEGATIVE” if at least one result is incorrect.



- Albania**, Institute of Public Health
- Austria** NRLP, Austrian agency for health and food safety
- Belgium** NRLP, Institute of Tropical Medicine
- Cyprus** NRLP, Veterinary Services
- Croatia** NRL, Croatian Veterinary Institute
- Czech Republic** NRLP, State Veterinary Institute
- Denmark** NRLP, Statens Serum Institut, laboratory of parasitology
- Estonia** NRLP, Animal Health, Veterinary and Food Laboratory
- Finland** NRLP, Finnish Food Authority, Ruokavirasto
- France** NRL Echinococcus, ANSES, LRFS Nancy
- Germany** NRL Echinococcus, Friedrich-Loeffler-Institut für Epidemiologie
- Greece** NRL Department of Parasitology-Parasitic Diseases Entomology & Bee Health
- Hungary** NRLP National Food Chain Safety Office, Laboratory of Parasitology, Fish and Bee Diseases
- Iceland** NRLP, Institute for Experimental Pathology Keldur
- Ireland** NRLP, Parasit section, Bact/Paras Division
- Italy** NRL, National reference laboratory for Echinococcosis, IZS della Sardegna
- Latvia** NRLP, Institute of food safety, animal health and environment
- Lithuanian** NRLP, National Food and Veterinary Risk Assessment Institute
- Northern Ireland**, AgriFood and Biosciences Institute (AFBI)
- Norway** NRLP, Norwegian Veterinary Institute
- Poland** NRLP, National Veterinary Research Institute
- Portugal** NRLP, Instituto nacional de investigacao agraria e veterinaria
- Republic of North Macedonia**, Faculty of Veterinary Medicine
- Romania** NRLP, Institute for diagnosis and animal health
- Slovakia** NRLP, Veterinary and Food Institute in Bratislava
- Slovenia** NRLP, University of Ljubljana, Veterinary Faculty
- Spain** NRLP, Laboratorio Central de Sanidad Animal
- Sweden** NRLP, National Veterinary Institute
- Switzerland**, Institute of Parasitology Vetsuisse Faculty, University of Bern
- The Netherlands** NRL, National Institute for Public Health and the Environment
- UK**, NRL for Trichinella and Echinococcus, Animal and Plant Health Agency,

# PARTICIPANTS (31)





# PT PREPARATION

- Carnivore intestines are collected and stored frozen at -80°C for one week.
- Faecal content is analysed by qPCR to exclude *E. multilocularis* infection.



Finnish Food authority Ruokavirasto, Finland



- The mucosa of the small intestine of foxes is collected, cleaned and sent to ISS.
- Mucosa is homogenised (with 70% ethanol; ratio 2:1), aliquoted, and spiked (double check).



PT panel:



25 worms



15 worms



0 worms

Worms have been previously deactivated (-80°C) by the providers.



Piwet, Poland

University of Zurich, Switzerland

Anses, France

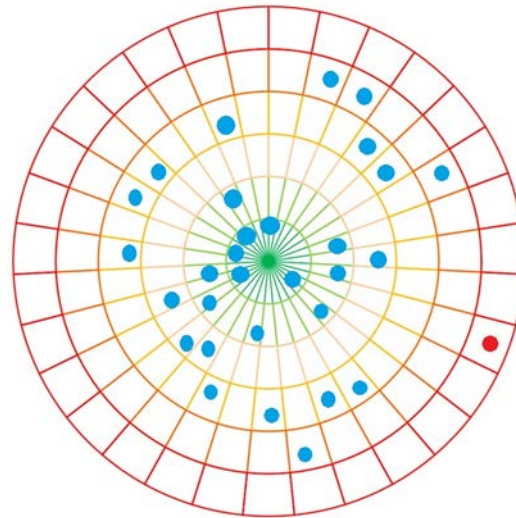
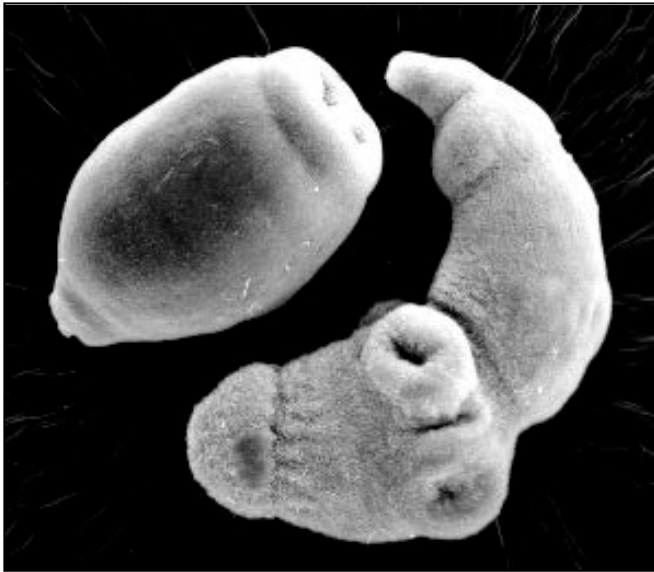
# SUMMARY OF RESULTS

|                                                       |                |
|-------------------------------------------------------|----------------|
| Number of participant laboratories submitting results | 31/31 (100%)   |
| Number of participants that passed the PT             | 26/31 (83.87%) |
| Number of participants that failed the PT             | 5/31 (16,13%)  |

1. False **negative**, 0 -> 25
2. False **negative**, 0 -> 15

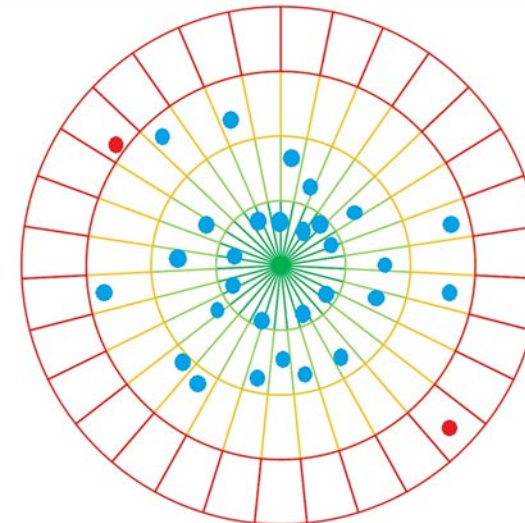
3. False **positive**, 1 -> 0
4. False **positive**, 8 -> 0

5. False **positive**, 8 -> 0  
& false **negative**, 0 ->15



Item1: 25

Mean of detection: 12

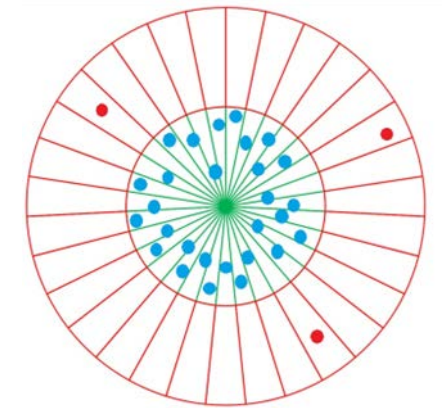


Item2: 15

Mean of detection: 11

1 over-estimation (21)

Without it, mean=10



Item3: 0

Mean of detection: 0,7

# ABOUT METHODOLOGY

- The most of the labs (26/31) used the Sedimentation and Counting Technique (SCT) with no relevant modification.
  - One lab (1/31) used the PBS instead of NaCl 0,9% (results were: 8, 10, 0 -> correct ✓ )
  - One lab (1/31) directly diluted the samples drop by drop in water under the binocular microscope (results were: 14, 10, 0 -> correct ✓ )
  - Three labs (3/31) poured the samples through a sieve
    - 180  $\mu$ M (results were: 21, 9, 0 -> correct ✓ )
    - 150  $\mu$ M (results were: 8, 10, 0 -> correct ✓ )
    - 50  $\mu$ M (results were: 18, 10, 0 -> correct ✓ )



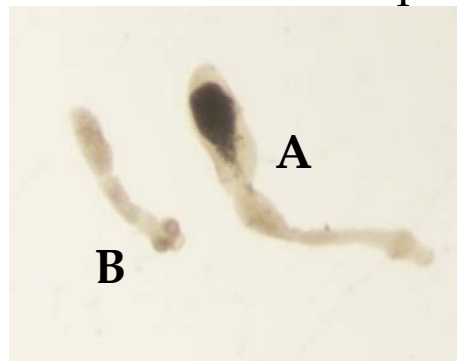
# ADDRESSING COMMENTS

- One lab observed **all** the worms floating in both positive items (no modification to SCT).
- Two labs observed Taeniidae worms different from *Echinococcus*. That's true ✓
- Two labs observed at least two different kind of *Echinococcus* (different in size) . That's true ✓

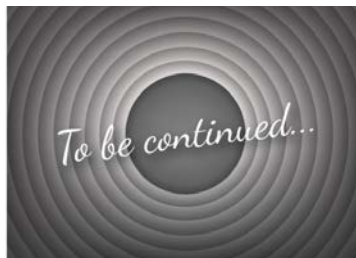


Reasoning:

- No single provision was sufficient for all items.
- It is necessary to maintain homogeneity across items.
- The three different batches were used in the same proportion, giving preference to the batch with larger worms. For example, out of 25 worms: 15 from Batch A, 5 from Batch B, and 5 from Batch C.



- Two labs observed Amobotaenia-like worms. That's true ✓



# AMOEBOTAENIA (?) -LIKE WORMS, AGAIN

- This year, the mucosa was naturally infected with cestode worms similar but different from *Echinococcus*.



Photos by Denise Hoek-van Deursen  
RIVM, The Netherlands

Photo by Famke Jansen  
ITM, Belgium

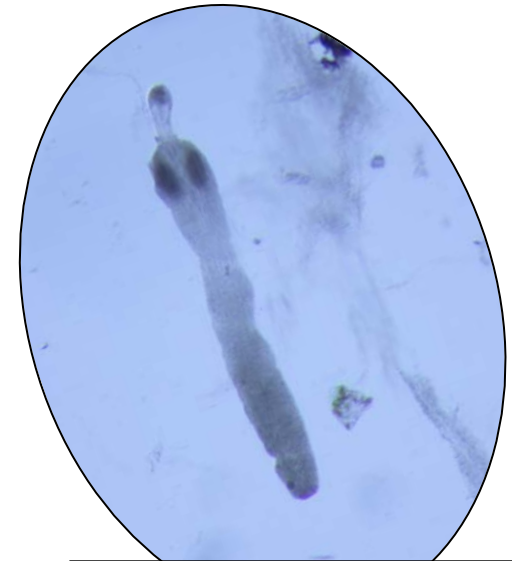


Photo by Federica Santolamazza  
EURLP, Italy

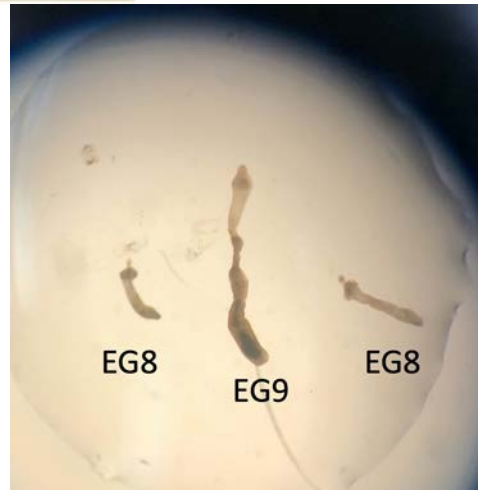
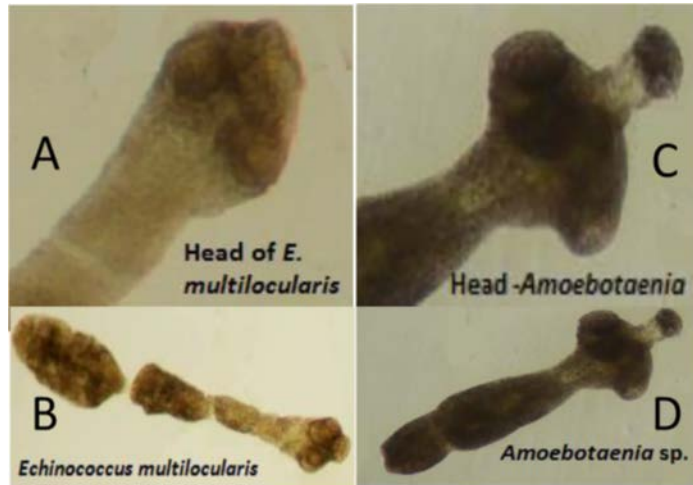
# AMOEBOTAENIA (?) -LIKE WORMS, AGAIN

- Similar occurrence in 2022-PT05.

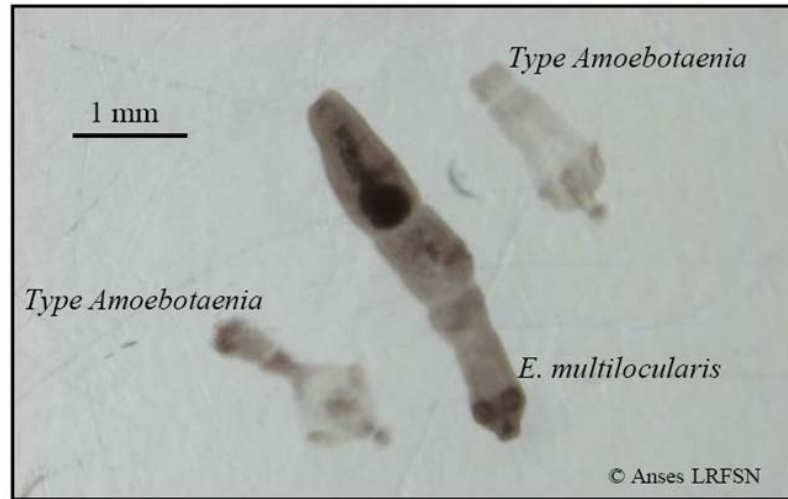


# AMOEBOTAENIA (?) -LIKE WORMS, AGAIN

At the time, we studied this new finding a bit.



Courtesy of F. Janssen



## Comparaison entre *E. multilocularis* et 2 cestodes de type *Amoebotaenia*

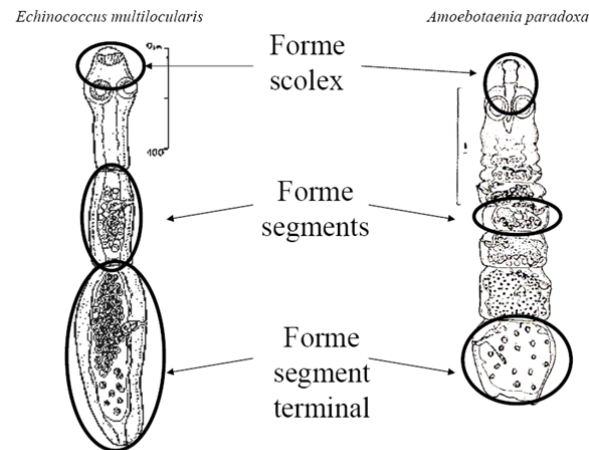


Schéma original d'*E. multilocularis* d'après Leuckart (1853) et schéma original d'*Amoebotaenia paradoxa* d'après Petavy et Deblock (1980).

Anses LRFSN  
LNR *Echinococcus* spp.  
Technopôle agricole et vétérinaire  
Bâtiment H  
54220 MALZEVILLE

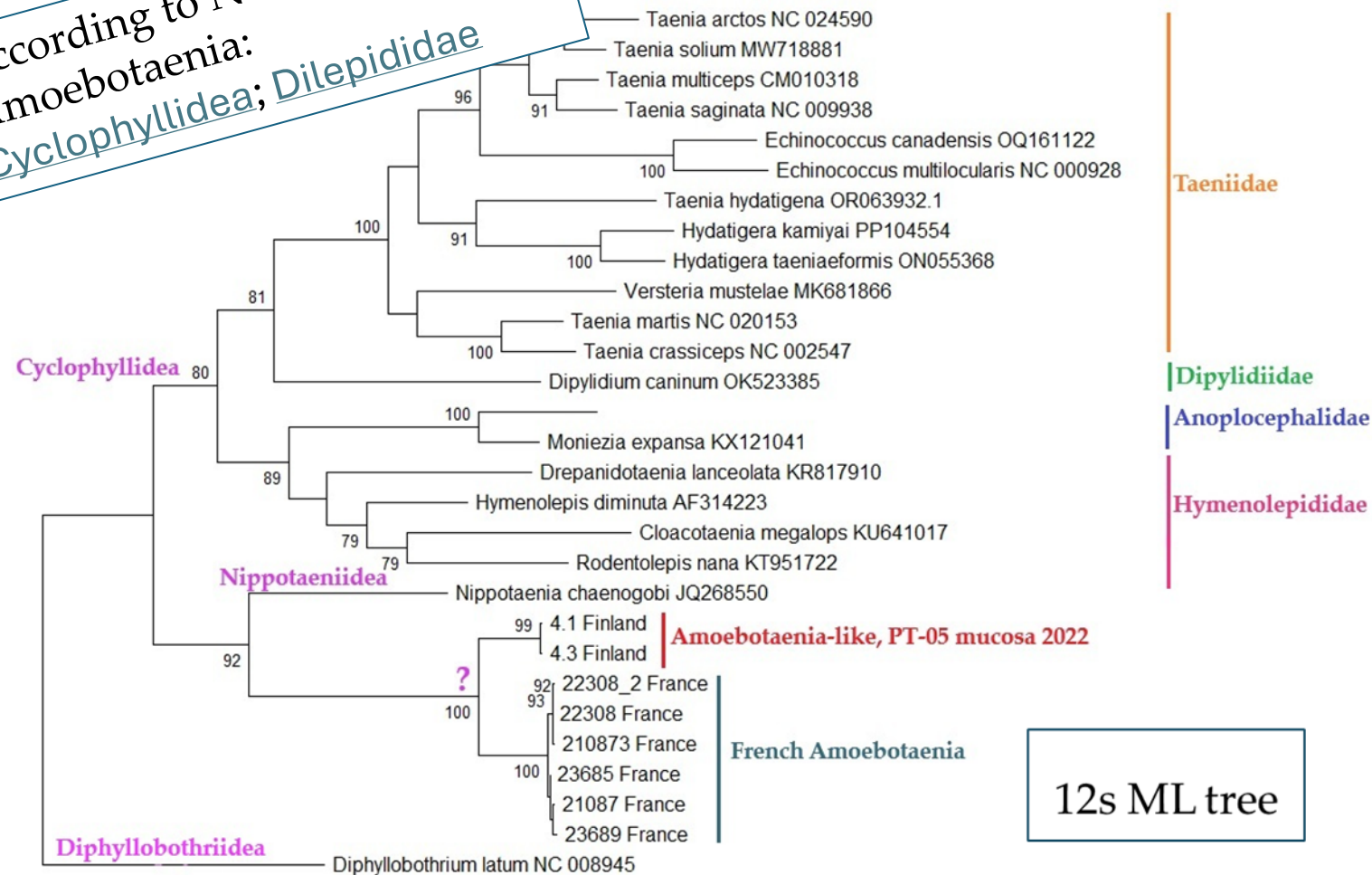
# AMOEBOTAENIA (?) -LIKE WORMS, AGAIN

- No confirmed

According to NCBI Taxonomy  
Amoebotaenia:  
Cyclophyllidae; Dilepididae

possible back then, as no *Amoebotaenia* spp sequences in GenBank (only a

them genetically.



2025:

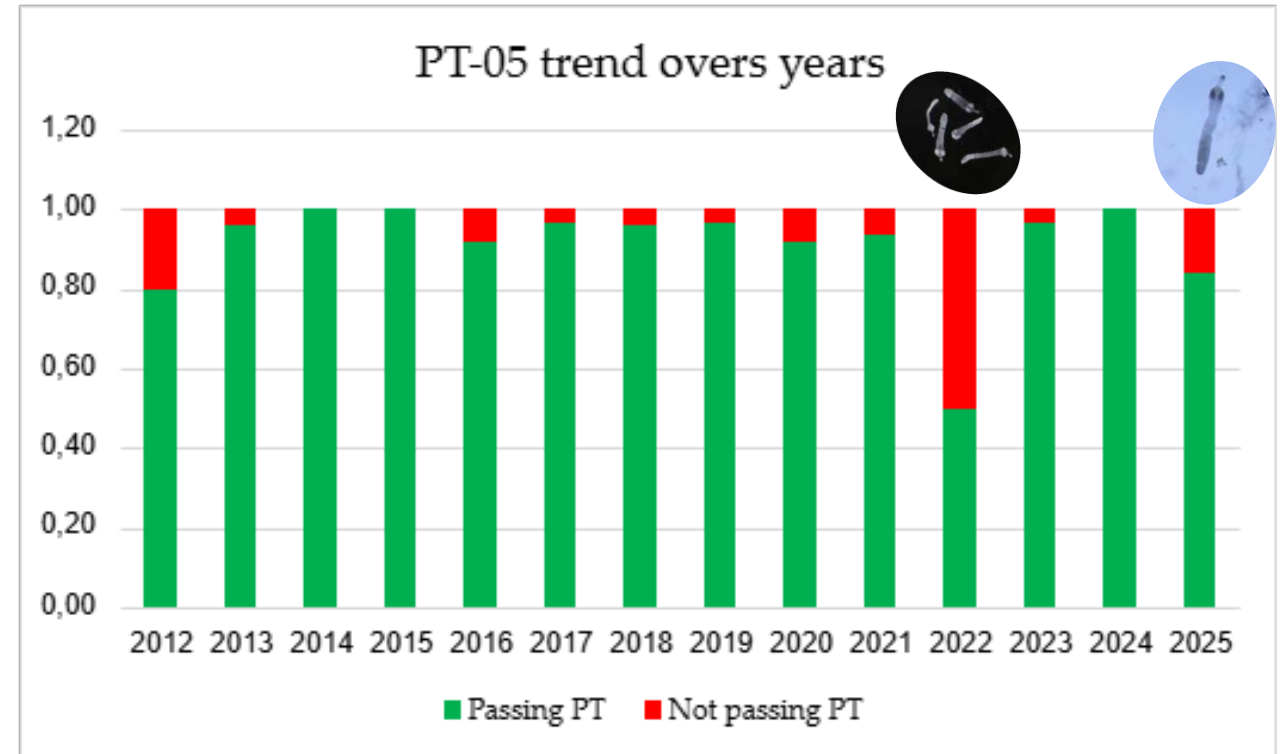


- 12s seminested: neg
- COX1 (JB3, JB4): neg
- NAD1 (KB11, JB12): neg
- Trachsel (Cest): neg
- Primers designed on old Amoebotaenias: neg
- Dilepididae 18S: neg

# 2025 HEATMAP OF RESULTS AND TREND OVER YEARS

|      |    |    |   |
|------|----|----|---|
| EM1  | 6  | 7  | 0 |
| EM2  | 4  | 5  | 0 |
| EM3  | 15 | 10 | 0 |
| EM4  | 9  | 5  | 0 |
| EM5  | 14 | 10 | 0 |
| EM6  | 11 | 7  | 0 |
| EM7  | 17 | 1  | 0 |
| EM8  | 10 | 7  | 0 |
| EM9  | 6  | 4  | 0 |
| EM10 | 18 | 12 | 0 |
| EM11 | 0  | 7  | 0 |
| EM12 | 23 | 11 | 8 |
| EM13 | 18 | 10 | 0 |
| EM14 | 15 | 11 | 0 |
| EM15 | 12 | 6  | 0 |
| EM16 | 4  | 6  | 0 |

|      |    |    |   |
|------|----|----|---|
| EM17 | 7  | 4  | 0 |
| EM18 | 8  | 10 | 0 |
| EM19 | 3  | 0  | 8 |
| EM20 | 1  | 1  | 0 |
| EM21 | 21 | 9  | 0 |
| EM22 | 20 | 11 | 0 |
| EM23 | 17 | 7  | 0 |
| EM24 | 10 | 4  | 0 |
| EM25 | 25 | 21 | 0 |
| EM26 | 20 | 9  | 0 |
| EM27 | 7  | 6  | 0 |
| EM28 | 9  | 4  | 0 |
| EM29 | 18 | 12 | 0 |
| EM30 | 11 | 6  | 1 |
| EM31 | 8  | 0  | 0 |



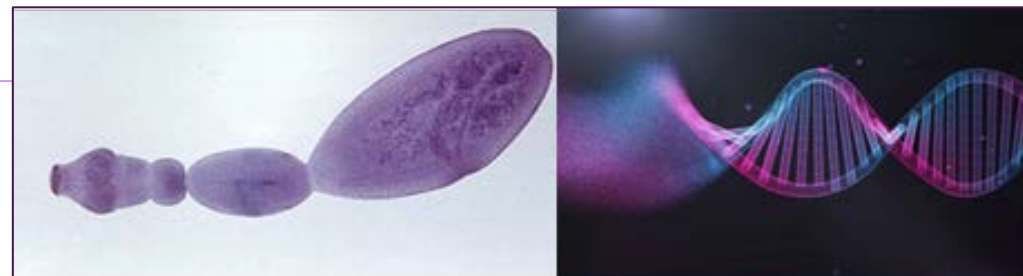
2022: 14/28 false positive results  
 2025: 3/31 false positive results  
 1/3 did not participate 2022 PT

Increased  
awareness?

## CONCLUSIONS

- In 2025, 26 out of 31 laboratories (84%) successfully passed PT-05.
- Detection performance appears robust, even when methods other than SCT are used.
  - The presence of natural infections with cestodes other than *Echinococcus* resulted in fewer errors compared to previous rounds, reflecting the increasing proficiency of NRL personnel.

# Molecular identification of *Echinococcus granulosus*, *Echinococcus multilocularis* and *Taenia spp*



XX Workshop of National Reference Laboratories for Parasites  
28<sup>th</sup> and 29<sup>th</sup> October 2025  
Istituto Superiore di Sanità

Federica Santolamazza, Azzurra Santoro

European Union Reference Laboratory for Parasites (EURLP);  
WHO Collaborating Centre for the Epidemiology, Detection and Control of Cystic and Alveolar Echinococcosis;  
ISTITUTO SUPERIORE DI SANITÀ (Rome, Italy)



## PT-08 Molecular identification of *E. granulosus*, *E. multilocularis* and *Taenia* spp.-2025

Due date to submit results: April 11, 2025

Individual report sent to participants within: April 30, 2025

Final report available on EURLP website from: May 31, 2025



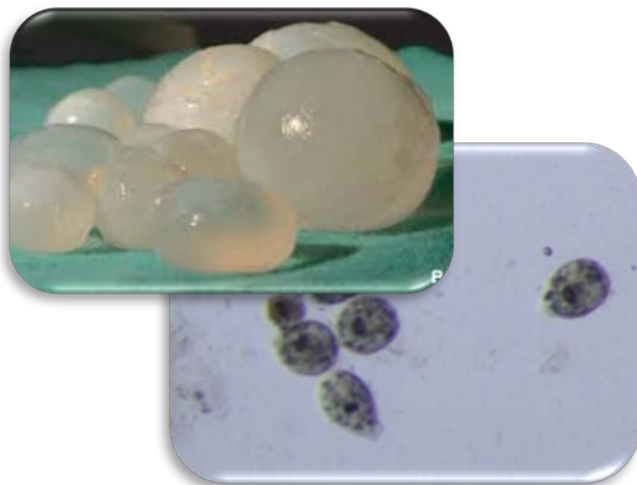
European Union Reference Laboratory for Parasites

# Aim of the PT

Evaluation of laboratories competence in molecular identification of

*Echinococcus granulosus sensu lato*,

*Echinococcus multilocularis* and *Taenia* spp





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# PT timing 2025



European Union Reference Laboratory for Parasites

## February 24th



PTs REQUEST FORM 2025

<https://www.iss.it/en/web/iss-en/eurlp-proficiency-testing>

## March 17<sup>th</sup>



## April 11<sup>th</sup>

# DEADLINE RESULT REPORTING

## April 30<sup>th</sup>

Individual Report PT-08

**Individual PT Report n. \_\_\_\_\_ Laboratory Code \_\_\_\_\_**

**PT "Detection of Anisakidae L3 larvae in fish fillets"**

Name \_\_\_\_\_  
Institution \_\_\_\_\_  
Address \_\_\_\_\_  
Tel: \_\_\_\_\_ Fax: \_\_\_\_\_ e-mail: \_\_\_\_\_

**Criteria for the result evaluation**  
The PT result evaluation is expressed as "correct" (right) or "incorrect" (false positive or false negative).  
The final evaluation is "positive" if the result is correct. The final evaluation is "negative" if at least one result is incorrect.

| SAMPLE CODE | N° of tested items | Result (N° of detected larvae) | Evaluation |
|-------------|--------------------|--------------------------------|------------|
|             |                    |                                |            |
|             |                    |                                |            |
|             |                    |                                |            |

**EVALUATION:**  
Recommendations: \_\_\_\_\_  
Date: \_\_\_\_\_ Head of EURLP Dr S.M. Cassi

**CONFIDENTIALITY:** The report (and its content) is the property of the EURLP. The EURLP reserves the right to provide, on request, the present PT result to the competent authority.

**End of the report**

PT Provider: Unit of Foodborne and Neglected Parasitic Diseases, Istituto Superiore di Sanità, Rome, Italy  
PT Coordinator: Dr. Anna Maria Cassi, Unit of Foodborne and Neglected Parasitic Diseases, Istituto Superiore di Sanità, Rome, Italy  
E-mail: [anacassi@iss.it](mailto:anacassi@iss.it) Tel: +39 06 4940 2070

## May 31<sup>st</sup>

**Final report PT-04: An 1/2021**

**PT-04: "Detection of Anisakidae L3 larvae in fish fillets"**

**Design**

|                          |                                                                                                                                                       |
|--------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|
| Purpose                  | Evaluation of laboratories in charge of official control on food                                                                                      |
| Scheme type              | Single, simultaneous                                                                                                                                  |
| Participants             | Public and private, European laboratories                                                                                                             |
| N° of participants       | Depending on request                                                                                                                                  |
| Method                   | Not regulated                                                                                                                                         |
| Test method              | Chosen by the participant                                                                                                                             |
| PT items                 | Matrix: 3 fish fillet sandwiches<br>Item: 3 for each participant<br>N° of samples: 3 for each participant<br>Preparation: Immediate after preparation |
| Subcontracted activities | None                                                                                                                                                  |
| Results evaluation       | Qualitative                                                                                                                                           |

**Logistics**

|                      |            |                        |                                                                               |
|----------------------|------------|------------------------|-------------------------------------------------------------------------------|
| Participants         | 30         | fish fillet sandwiches | 63                                                                            |
| Private laboratories | 2          | PT panel composition   | 3 fish fillet sandwiches<br>one spiked with 1 larva, two spiked with 3 larvae |
| NRL                  | 28         | Shipping               | CHL                                                                           |
| Shipping dates       | 15/03/2021 |                        |                                                                               |

PT Provider: Unit of Foodborne and Neglected Parasitic Diseases, Istituto Superiore di Sanità  
Via Regina Elena, 289 - 00158 Rome, Italy  
PT Person in charge: Dr. Maria Lilla  
E-mail: [marilla@iss.it](mailto:marilla@iss.it) Tel: +39 06 4940 2070

ACCREDIS  
FTP N° 0005 P  
Materiale degli Accordi di Mutuo Riconoscimento  
RA, SAP e SAE  
Seguono di RA, SAP e SAE  
Mutuo Riconoscimento



## PT-08 Molecular identification of *E. granulosus*, *E. multilocularis* and *Taenia* spp.-2025

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European Union Reference Laboratory for Parasites

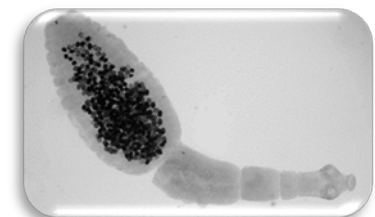
# Preparation of samples PT08-2025

The PT has been organized following the NRL request (2025)

The panel consists of 4 tubes:

3 tubes containing DNA extracted from canine faecal stool **spiked** with:

- *E. granulosus* s.s. cyst
- *E. multilocularis* worms
- *Taenia hydatigena* proglottid



Negative: *Anisakis* DNA



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European Union Reference Laboratory for Parasites

# Detection method PT08-2025

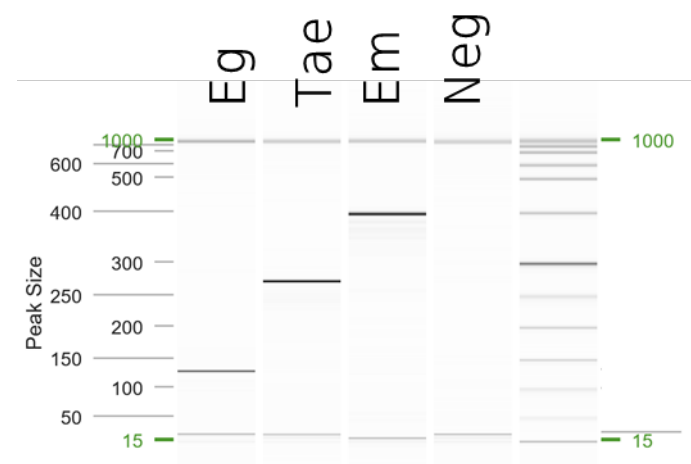
The samples were identified at species level through multiplex PCR

***E.g.* = *E. granulosus* (117 bp)**

***E.m.* = *E. multilocularis* (395 bp)**

**Tae= *Taenia* spp (267bp)**

**Negative= *Anisakis* spp**



**Multiplex PCR** (Trachsel *et al.* 2007)

In 30  $\mu$ l total reaction with 0.2 mM of each primer (Cest1, Cest2, Cest3, Cest4, Cest5)

**Dilution 1:10 and 1:100**

**COX1 PCR and sequencing (366bp)**



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# Detection method PT08-2025

**Homogeneity** was ensured by providing participants with aliquots of the same DNA preparation.

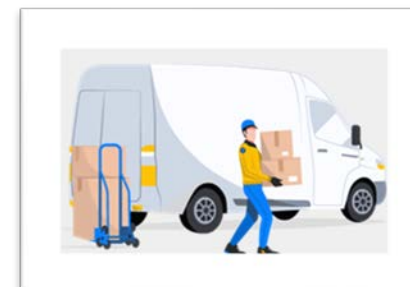


The tubes were plugged and sealed using plastic parafilm, individually coded.



Each PT panel was inserted in polystyrene box with ice pack

All panels were delivered within 24-36 hours.





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# Evaluation criteria

The PT evaluation is qualitative, and no statistical analysis of the results is applied

| Lab code | Expected                                                                           | Observed                                                                           | Result (correct/incorrect)                   | Evaluation (positive/negative) |
|----------|------------------------------------------------------------------------------------|------------------------------------------------------------------------------------|----------------------------------------------|--------------------------------|
| EGM X    | <i>E. granulosus</i><br>Negative<br><i>Taenia</i> spp.<br><i>E. multilocularis</i> | <i>E. granulosus</i><br>Negative<br><i>Taenia</i> spp.<br><i>E. multilocularis</i> | correct<br>correct<br>correct<br>correct     | Positive                       |
| EGM X    | <i>E. granulosus</i><br>Negative<br><i>Taenia</i> spp.<br><i>E. multilocularis</i> | <i>E. granulosus</i><br><i>Taenia</i> spp.<br>Negative<br><i>E. multilocularis</i> | correct<br>incorrect<br>incorrect<br>correct | Negative                       |
| EGM X    | <i>E. granulosus</i><br>Negative<br><i>Taenia</i> spp.<br><i>E. multilocularis</i> | <i>E. granulosus</i><br>Negative<br><i>Taenia</i> spp.<br>Negative                 | correct<br>correct<br>incorrect<br>correct   | Negative                       |

The result is “correct” if the PT items are correctly identified

The result is “incorrect” if the PT items are incorrectly identified

The PT is considered “POSITIVE” if the results of all samples are “correct”

The PT is considered “NEGATIVE” if at least one result is “incorrect”



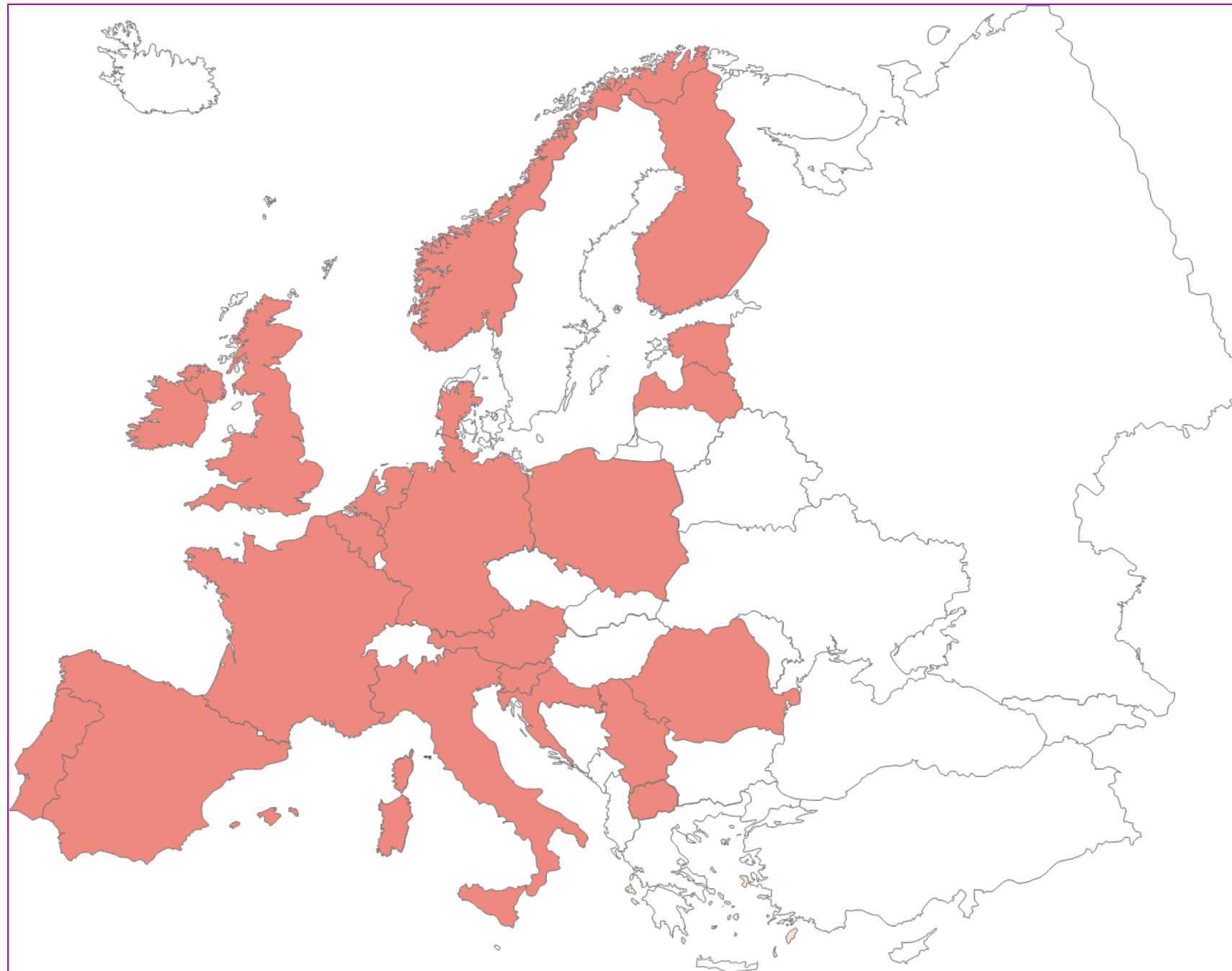
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## Participants (22)



Austria  
Belgium  
Croatia  
Denmark  
Estonia  
Finland  
France  
Germany  
Ireland  
Italy  
Italy (2° lab)  
Latvia  
Norway  
Poland  
Portugal  
Rep. North Macedonia  
Romania  
Slovenia  
Spain  
Switzerland  
The Netherlands  
UK



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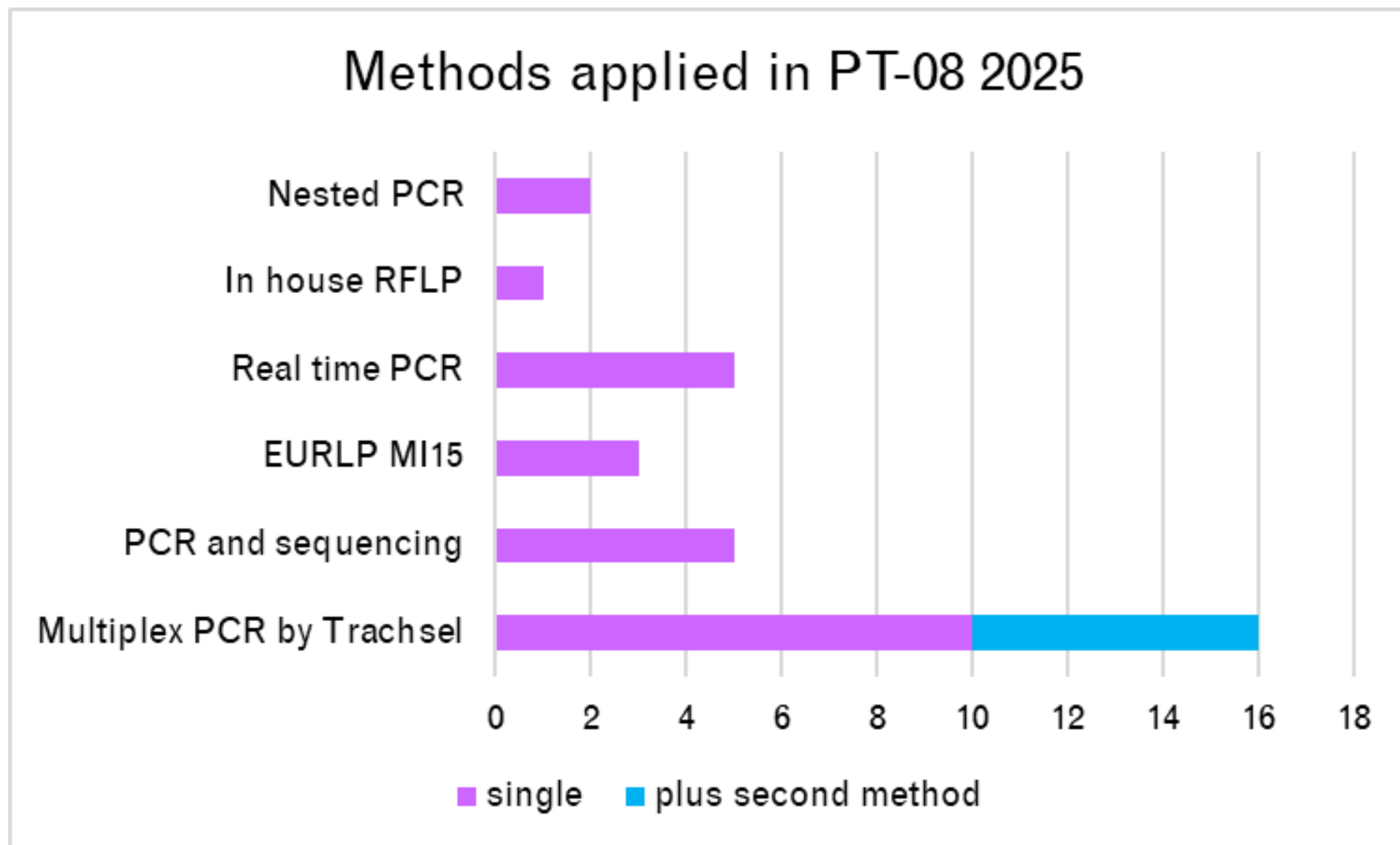
Final report available on EURLP website from: May 31, 2025



Funded by the  
European Union

# Methods

The EURLP recommends the use of the multiplex PCR by Trachsel *et al.* however, any other suitable molecular-based method is accepted





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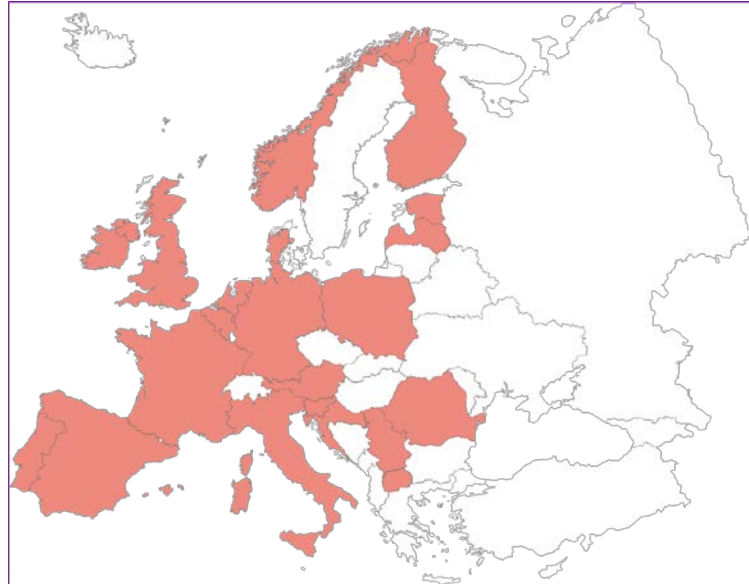
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# Results



**Participants N=22**

**Participants passed N=20**

**Participants failed N=2**

Sample1 (*E. granulosus* s. l.): 22 labs (**100%**) out of 22 obtained a positive evaluation

Sample 2 (negative): 21 labs (95%) **1. false positive** -> the lab used the evaluation  
Multiplex PCR by Trachsel, 2007

Sample 3 (*Taenia* spp.): 21 labs (90%) **2, false negative** -> the lab used the evaluation  
the Multiplex by Trachsel, 2007 and the nad1 PCR & sequencing.

Sample 4 (*E. multilocularis*): 22 labs (100%) **3. false negative** -> the lab used the evaluation  
The lab reported that they did not include primers for *Taenia* spp.

**Carefully read emails, procedure  
and instructions!**

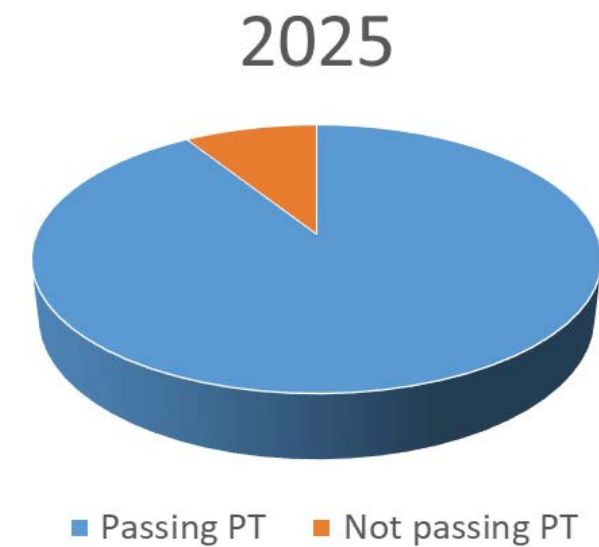


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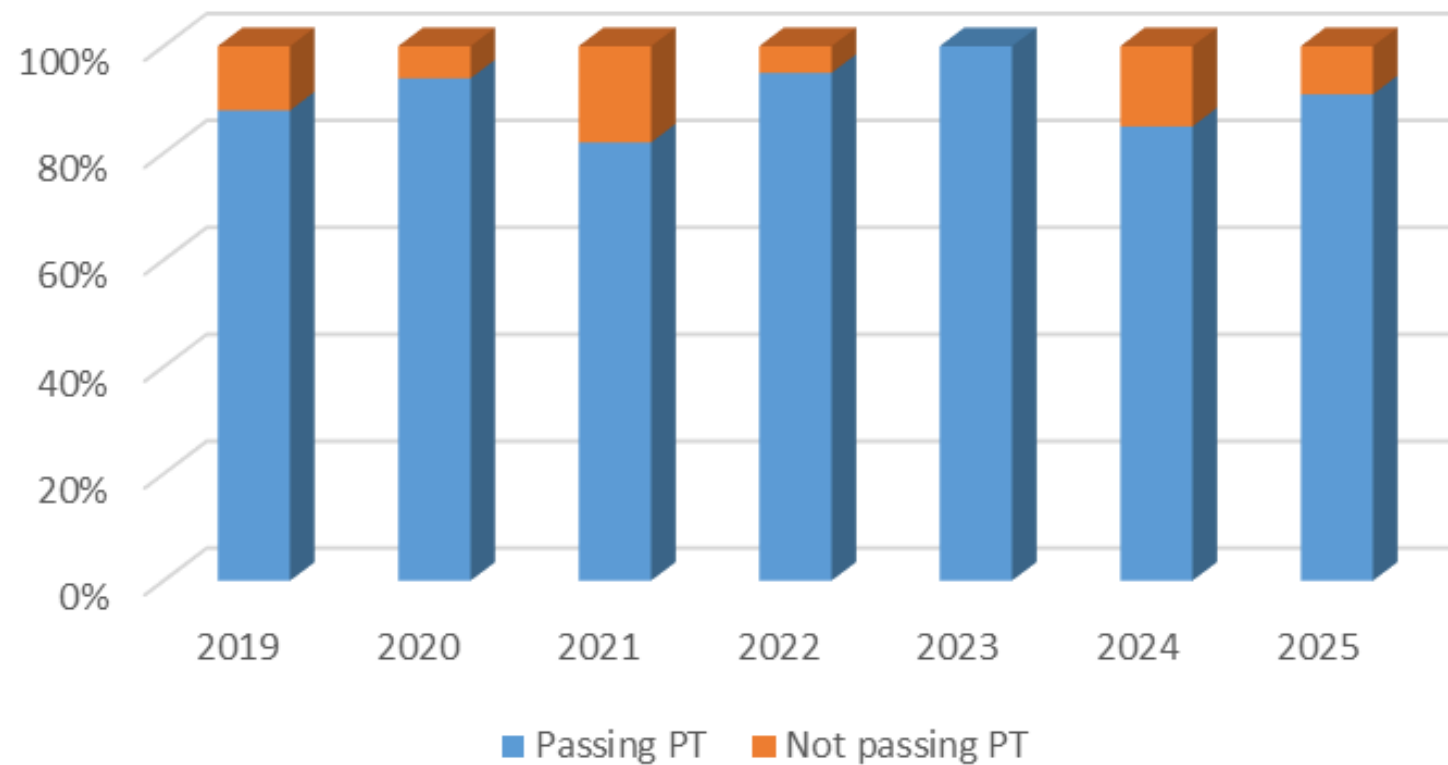
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 Final report available on EURLP website from: May 31, 2025



European Union Reference Laboratory for Parasites



### PT-08 Trend overs years





## PT-08 Molecular identification of *E. granulosus*, *E. multilocularis* and *Taenia* spp.-2025

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# Suggestions, based on this year's experience:

- Species identified during the Proficiency Test:  
defined by:

### Procedure

#### PT items

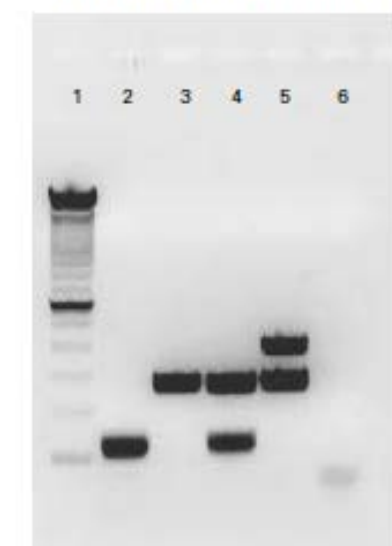
Description. The PT panel consists of four tubes containing: DNA extracted from faecal material contaminated with *Echinococcus granulosus sensu lato* (s.l.), DNA extracted from faecal material contaminated with *Echinococcus multilocularis*, DNA extracted from faecal material contaminated with *Taenia* spp. and a negative control.

- Mixed-DNA items?

Not so far.

However, this could be a possible upgrade of the PT.  
If implemented, the procedure will be updated.

D. Trachsel, P. Deplazes and A. Mathis



Trachsel et al., 2007



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## Conclusions:

Out of 22 laboratories, 20 successfully passed the PT.

No specific errors were identified.

Overall, all the chosen methods appear to perform well, and NRL personnel demonstrate sufficient expertise to handle the PT-08, even when new challenges are introduced.



# Thanks for your attention



## ACKNOWLEDGMENTS

Federica Santolamazza  
Alessia Possenti



**EURLP**