Istituto Superiore di Sanità
Department of Infectious Diseases
Unit of Foodborne and Neglected Parasitic Diseases
European Union Reference Laboratory for Parasites

4th PROFICIENCY TESTING

"Molecular identification of *Echinococcus* spp. at the species level"

XVII Workshop of National Reference Laboratories for Parasites
15th and 16th september 2022
Istituto Superiore di Sanità

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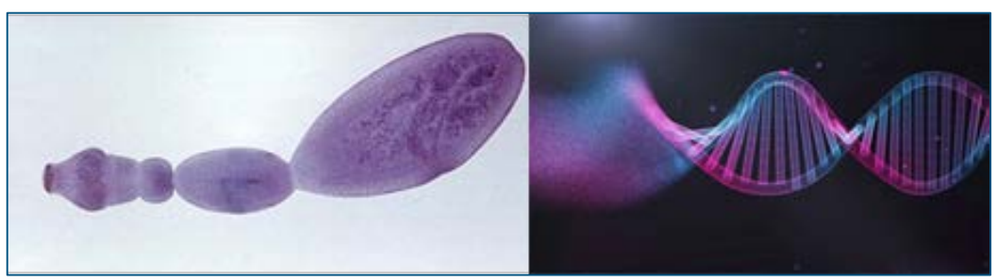
WHO Collaborating Centre for the Epidemiology, Detection and Control of Cystic and Alveolar Echinococcosis;
ISTITUTO SUPERIORE DI SANITÀ (Rome, Italy)

PT-08 Molecular identification of Echinococcus at the species level-2022

Due date to submit results: April 15, 2022
Individual report sent to participants within: May 2, 2022
Final report available on EURLP website from: May 31, 2022



Aim of the PT

Evaluation of laboratories competence in molecular identification of

Echinococcus granulosus sensu latu

and

Echinococcus multilocularis

PT timing 2022

January 25th

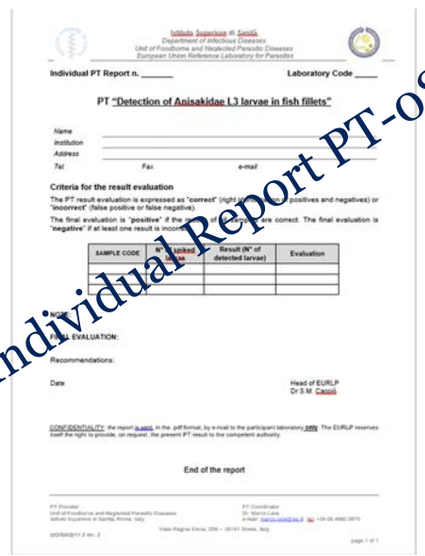
PTs REQUEST FORM 2022

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

March 14th

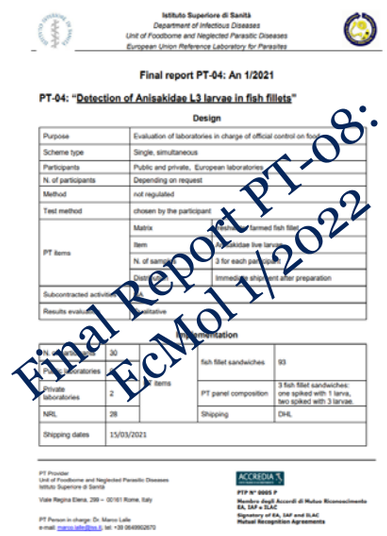
April 15th

April 29th

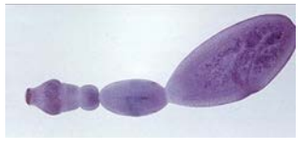


Individual Report PT-08

May 31st



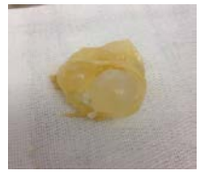
Final Report PT-08: EcMol 1/2022



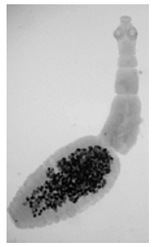
Preparation of samples PT08-2022

PT panel consists of 3 tubes, each containing 10 µl DNA of *E. granulosus s.s.*, *E. multilocularis*, and *Taenia* spp.

Echinococcus granulosus cysts were collected from human hosts

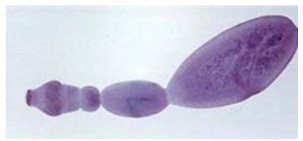


Echinococcus multilocularis worms were collected from foxes



Taenia spp. worms were collected from canids

The samples were stored in 70% ethanol
A commercial kit was used for the DNA extraction



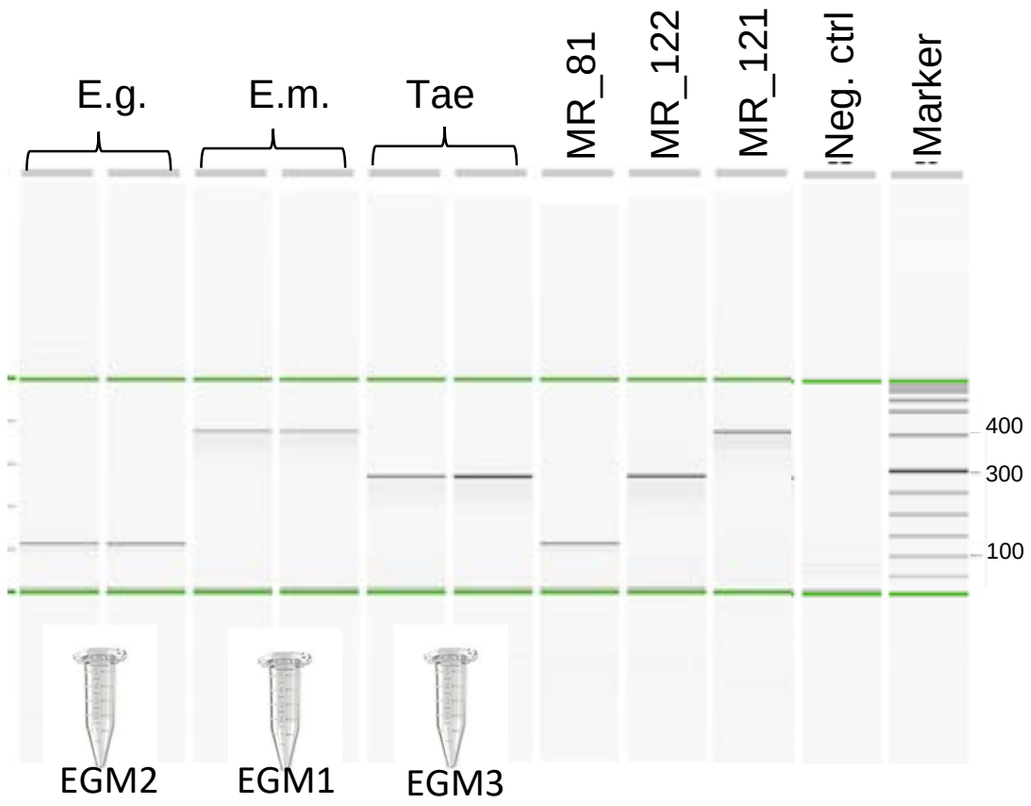
Detection method PT08-2022

The samples were individually identified at species level through multiplex PCRs

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

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

Tae = *Taenia saginata* (267 bp)



Reference Material EURLP

MR_81 = *E. granulosus* (G3)

MR_121= *E. multilocularis*

MR_122 = *Taenia saginata*

Multiplex PCR (Trachsel *et al.* 2007)
In 30 µl total reaction with 0.2 mM of primer (Cest1, Cest2, Cest3, Cest4, Cest5)

Preparation of samples PT08-2022

Homogenity 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 polystirene box with ice pack.

All panels were delivered within 24-36 hours

Evaluation criteria

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

The result is "correct" if the PT items are correctly identified

The result is "incorrect" if PT items are incorrectly identified

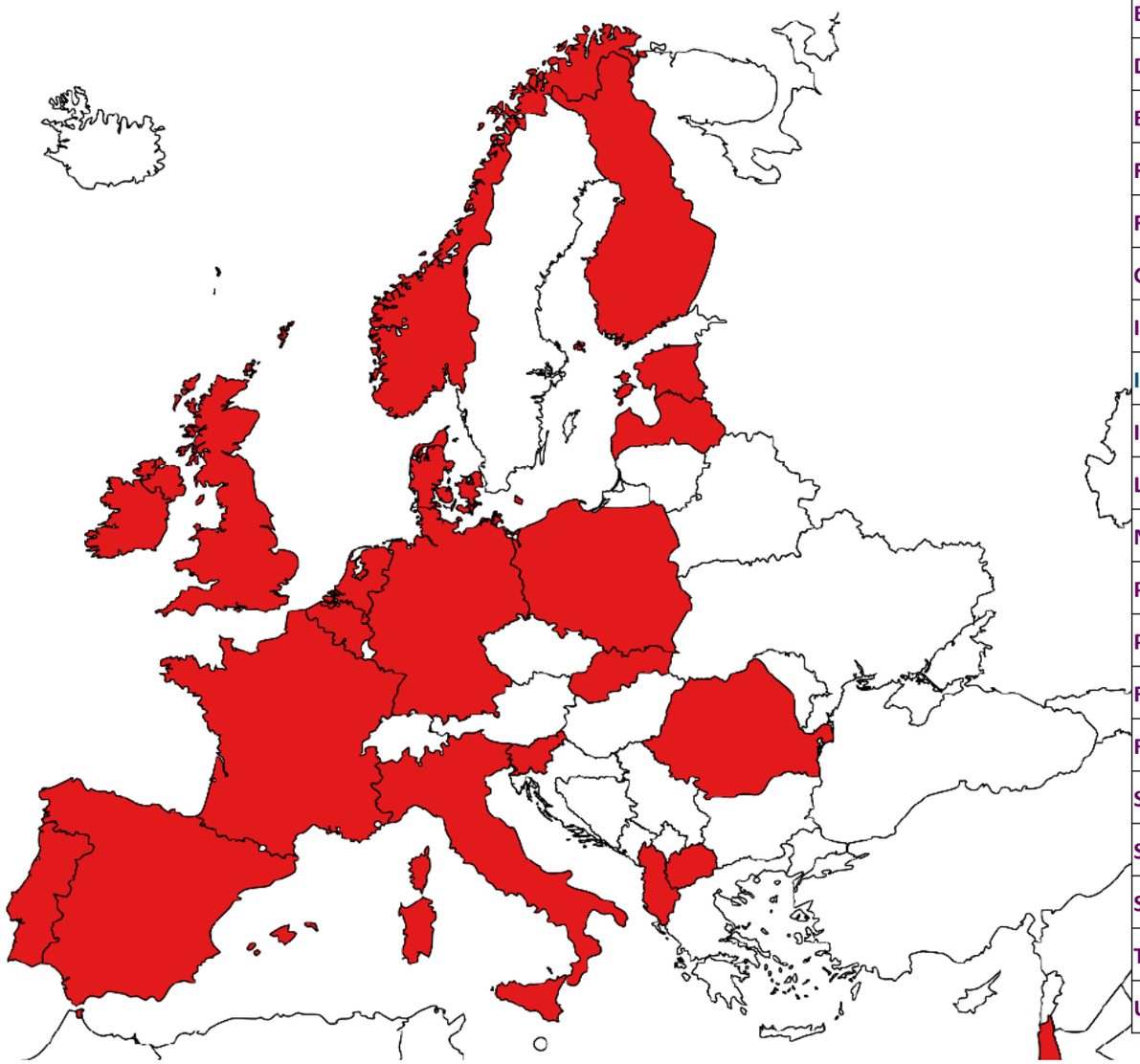
Lab code	Expected	Observed	Result (correct/incorrect)	Evaluation (positive/negative)
Ed x	Eg	Eg	correct	Positive
	Em	Em	correct	
	neg	neg	correct	
Ed xx	Eg	Eg	correct	Negative
	Em	Em	correct	
	neg	Eg	incorrect	
Ed xxx	Eg	Em	incorrect	Negative
	Em	Em	correct	
	neg	neg	correct	

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"

PT-08 Molecular identification of Echinococcus at the species level-2022

RESULTS

Participants (N=20)
20 labs submitted results



Belgium NRLP, Institute of Tropical Medicine
Denmark NRLP, Statens Serum Institut, laboratory of parasitology, SSI
Estonian NRLP, Animal Health, Veterinary and Food Laboratory
Finland NRLP, Oulu, Finnish Food Authority, Ruokavirasto (ex Evira)
France NRL Echinococcus, ANSES, LRFS Nancy
Germany NRL Echinococcus, Friedrich-Loeffler-Institut fur Epidemiologie
Ireland NRLP, Parasit section, Bact/Paras Division, Backweston Campus, Celbridge Kildare
Israel, Division of Parasitology, Kimron Veterinary Institute, Israeli Veterinary Services
Italy NRL Echinococcus, IZS Istituto Zooprofilattico Sperimentale della Sardegna
Latvia NRLP, Institute of food safety, animal health and environment, BIOR
Norway NRLP, Norwegian Veterinary Institute
Poland NRLP, National Veterinary Research Institute , Department of Parasitology and Invasive Diseases
Portugal NRLP, Instituto nacional de investigacao agraria e veterinaria
Republic of North Macedonia, Faculty of Veterinary Medicine, Skopje
Romania NRLP, Institute for diagnosis and animal health
Slovak Republic NRLP, Veterinary and Food Institute in Bratislava
Slovenian NRLP, University of Ljubljana, Veterinary Faculty
Spain NRLP, Laboratorio Central de Sanidad Animal
The Netherlands NRLP, National Institute for Public Health and the Evironment (RIVM)
UK NRL for Trichinella and Echinococcus, Animal and Plant Health Agency, York

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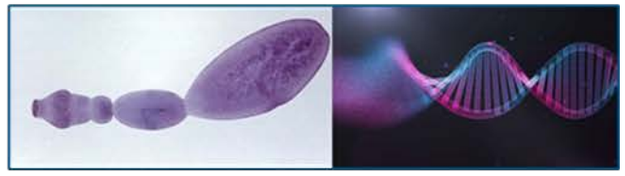
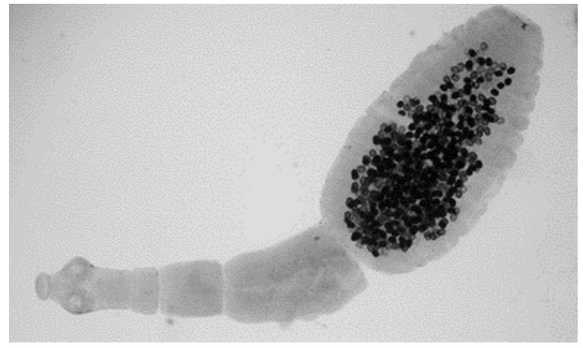


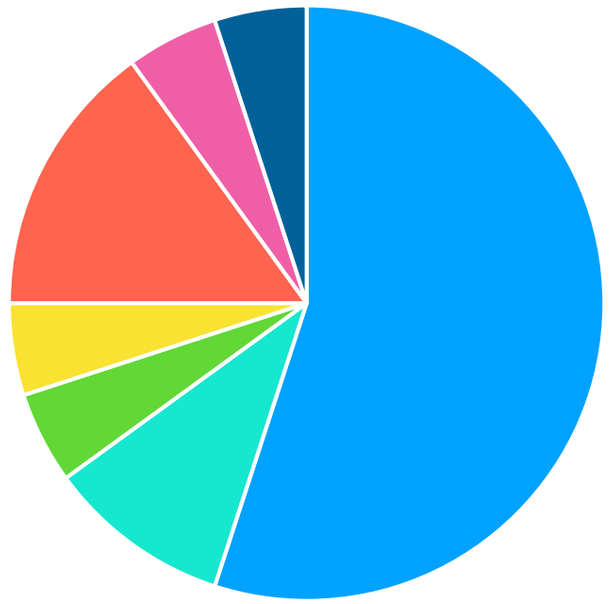
RESULTS

Detection method

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



Method applied	N. labs
Trachsel *et al*., 2007	11
Bowles *et al*., 1992	2
Geysen *et al*., 2007	1
Laurimaa *et al.,* 2015	1
EURLP method+ sequencing	3
OIE Terrestrial Manual 2019	1
In house method: CO1 PCR and sequencing	1



PT-08 Molecular identification of Echinococcus at the species level-2022

RESULTS

Number of participant laboratories	**20**
Number of participants that passed the PT	19
Number of participants that failed the PT	1

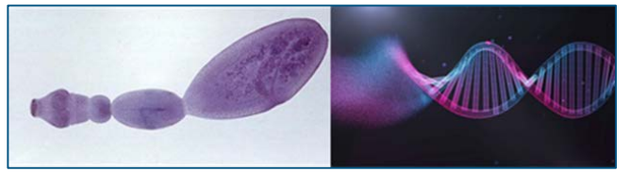
Sample 1 - *E. multilocularis*: 19 labs (95%) out of 20 obtained a positive evaluation

Sample 2 - *E. granulosus s.l.*: 20 labs (100%) out of 20 obtained a positive evaluation

Sample 3 - *Taenia saginata*: 20 labs (100%) out of 20 obtained a positive evaluation

PT-08 Molecular identification of Echinococcus at the species level-2022

RESULTS



Lab code	Code	Expected	Observed	Results	Evaluation (Positive/Negative)	Lab code	Code	Expected	Observed	Results	Evaluation (Positive/Negative)
Ed1	EGM1 EGM2 EGM3	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE	**Ed11**	EGM31 EGM32 EGM33	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE
Ed2	EGM4 EGM5 EGM6	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE	**Ed12**	EGM34 EGM35 EGM36	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE
Ed3	EGM7 EGM8 EGM9	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE	**Ed13**	EGM37 EGM38 EGM39	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE
Ed4	EGM10 EGM11 EGM12	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE	**Ed14**	EGM40 EGM41 EGM42	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE
Ed5	EGM13 EGM14 EGM15	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE	**Ed15**	EGM43 EGM44 EGM45	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE
Ed6	EGM16 EGM17 EGM18	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE	**Ed16**	EGM46 EGM47 EGM48	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE
Ed7	EGM19 EGM20 EGM21	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE	**Ed17**	EGM49 EGM50 EGM51	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE
Ed8	EGM22 EGM23 EGM24	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE	**Ed18**	EGM52 EGM53 EGM54	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE
Ed9	EGM25 EGM26 EGM27	E. multilocularis E. granulosus sl Negativo	E. multilocularis and Taenia spp E. granulosus sl Negative	Incorrect Correct Correct	**NEGATIVE**	**Ed19**	EGM55 EGM56 EGM57	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE
Ed10	EGM28 EGM29 EGM30	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE	**Ed20**	EGM58 EGM59 EGM60	E. multilocularis E. granulosus sl Negativo	E. multilocularis E. granulosus sl Negativo	Correct Correct Correct	POSITIVE

Conclusions

The experience derived from the fourth PT carried out in 2022 on the molecular detection of *Echinococcus* spp. showed that the personnel of NRLs are skilled to detect this parasite in a qualitative test

95% of participants succeeded in the identification of *Echinococcus multilocularis*, 100% of participants correctly identified *Echinococcus granulosus s. l.* and 100% detected the negative sample

Recommendations:

We suggest the laboratories to optimize multiplex PCR settings to avoid unspecific amplifications and to sequence amplicons to confirm the identification

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