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

XVIII Workshop of National Reference Laboratories for Parasites
16th and 17th November 2023
Istituto Superiore di Sanità

5th PROFICIENCY TESTING

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

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ISTITUTO SUPERIORE DI SANITÀ (Rome, Italy)

Istituto Superiore di Sanità
Department of Infectious Diseases
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European Union Reference Laboratory for Parasites

Aim of the PT

PT-08 Molecular identification of Echinococcus at the species level-2023

Due date to submit results: April 14, 2023
Individual report sent to participants within: May 1, 2023
Final report available on EURLP website from: May 31, 2023

Evaluation of laboratories competence in molecular identification of
Echinococcus granulosus sensu latu and
Echinococcus multilocularis

PT timing 2023

January 10th

PTs REQUEST FORM 2023

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

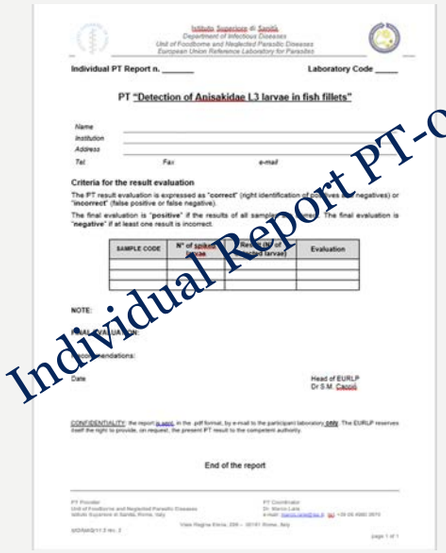
March 13th

April 14th

DEADLINE

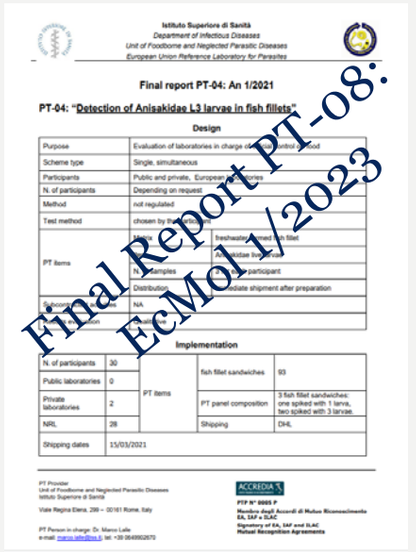
RESULT REPORTING

May 1st



Individual Report PT-08

May 31st



Final Report PT-08: EcMoH/2023

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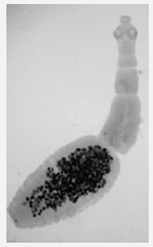
Preparation of samples PT08-2023

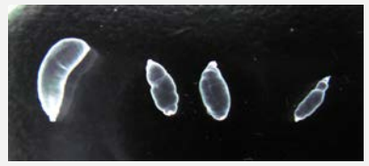
PT panel consists of 3 tubes, each containing 10 µl DNA of *E. granulosus s.s.*, *E. multilocularis*, and *Taenia* spp.

Echinococcus granulosus cysts were collected from human hosts



Echinococcus multilocularis worms were collected from foxes

Taenia crassiceps metacestode were collected from rodent



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

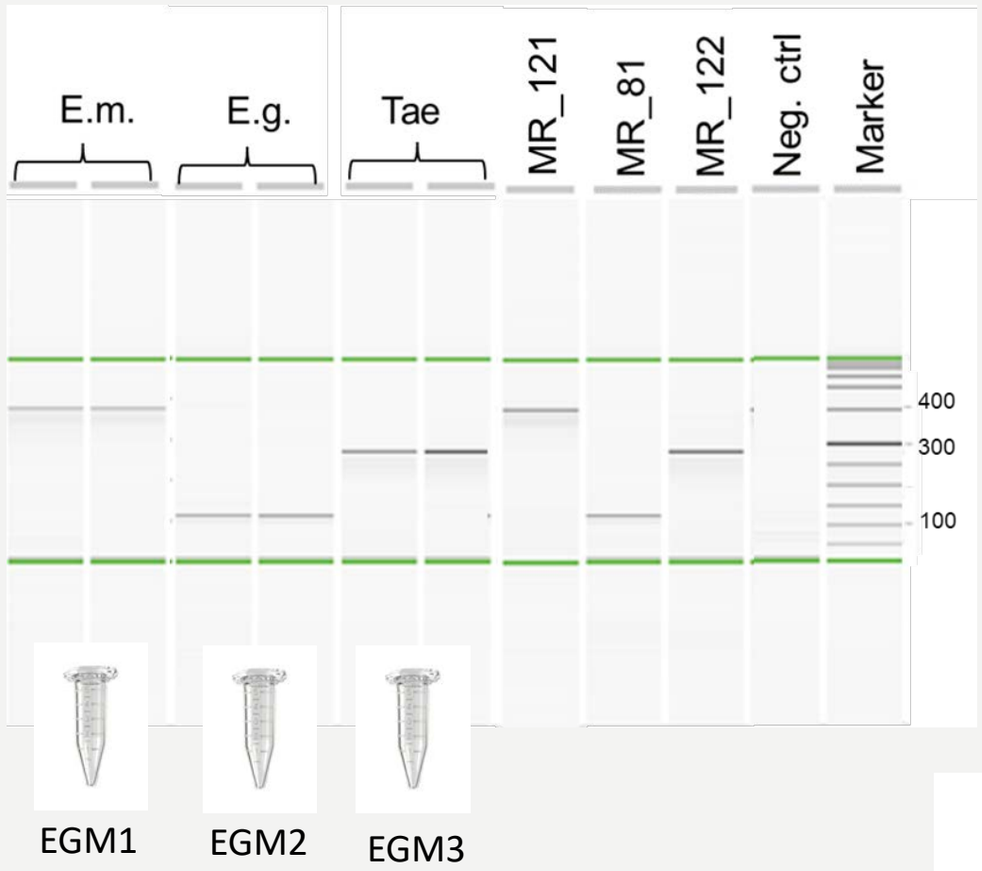
Detection method PT08-2023

The samples were individually identified at species level through multiplex PCR

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

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

Tae = *Taenia spp* (267 bp)



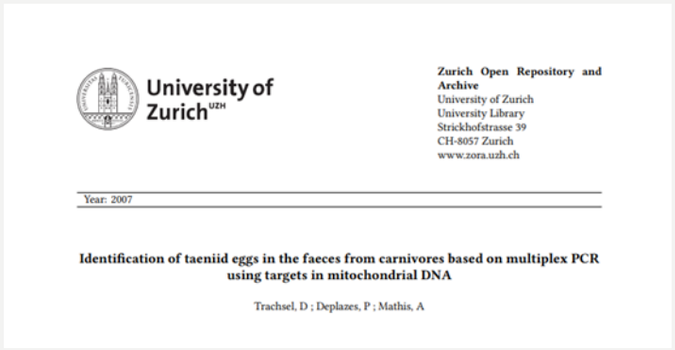
Reference Material EURLP

MR_121= *E. multilocularis*

MR_81 = *E. granulosus* (G3)

MR_122 = *Taenia crassiceps*

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

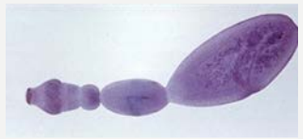

University of Zurich[UZH]

Zurich Open Repository and Archive
University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2007

Identification of taeniid eggs in the faeces from carnivores based on multiplex PCR using targets in mitochondrial DNA

Trachsel, D ; Deplazes, P ; Mathis, A



Test material

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 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	
	Eg	neg	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-2023

Due date to submit results: April 14, 2023
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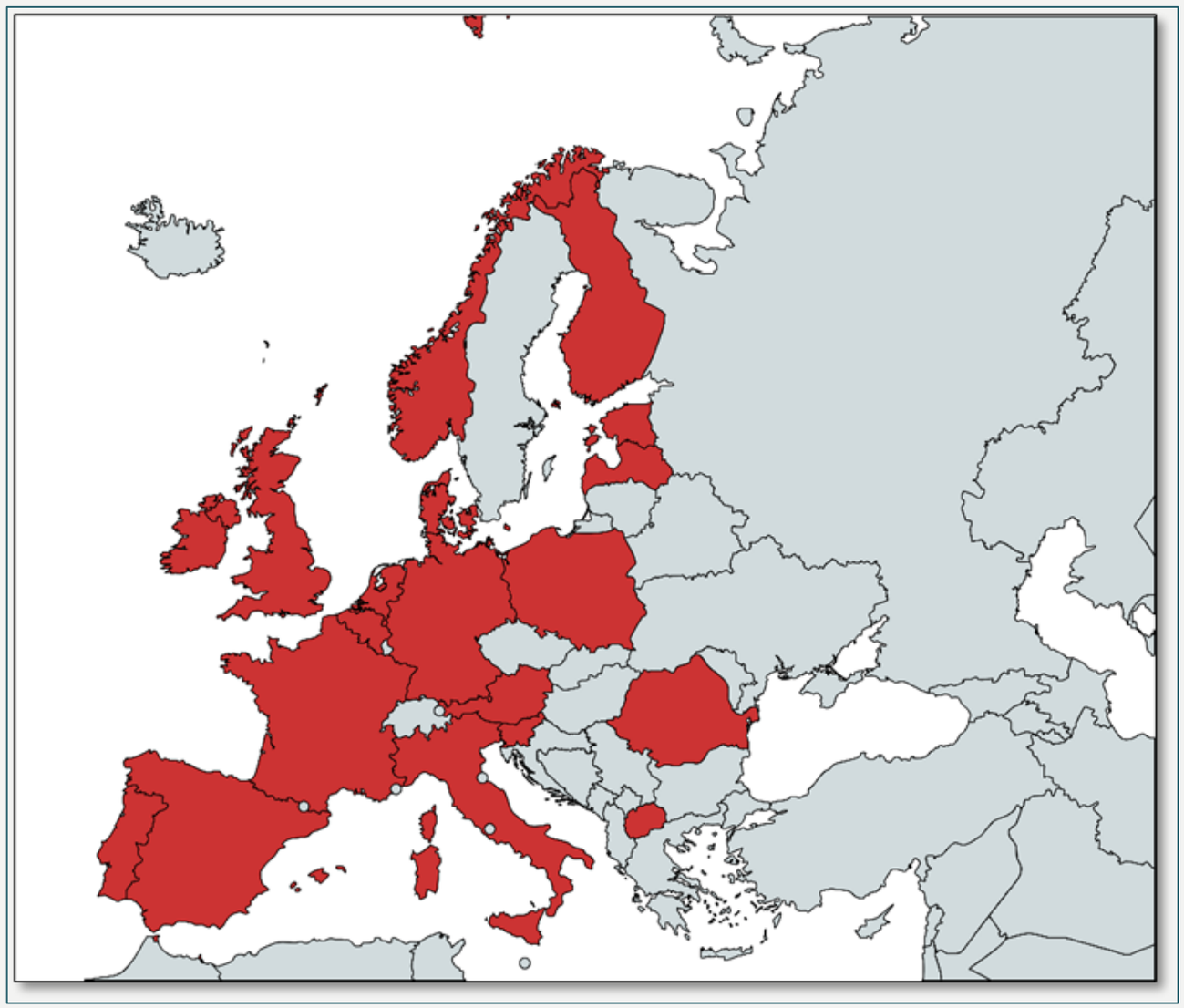
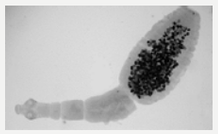
Participants (N=19)
19 labs submitted results



Belgium NRLP, Institute of Tropical Medicine
Denmark NRLP, Statens Serum Institut, laboratory of parasitology
Estonian NRLP, Animal Health, Veterinary and Food Laboratory
Finland NRLP, Oulu, Finnish Food Authority, Ruokavirasto
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
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



Detection method

The EURLP recommends the use of the multiplex PCR assay developed by Trachsel *et al*.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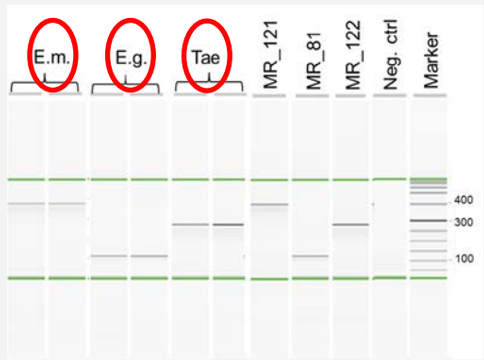
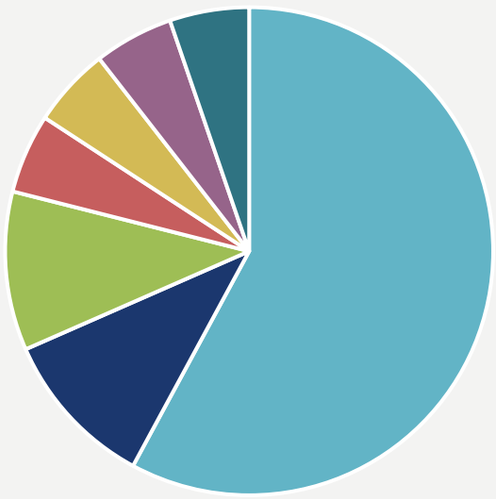


Method applied	N. labs
Multiplex PCR (Trachsel *et al*., 2007)	11
EURLP method	2
EURLP method + in house PCR + sequencing	2
EURLP method + Real Time PCR (Øines et al., 2014)	1
Semi-nested PCR+ sequencing (Geysen *et al*., 2007)	1
PCR_RFLP (in house method)	1
PCR (in house method) + sequencing	1



RESULTS

Number of participant laboratories	**19**
Number of participants that passed the PT	19
Number of participants that failed the PT	0

Sample **1** (EGM1)- *E. multilocularis*: 19 labs (100%) out of 19 obtained a positive evaluation

Sample **2** (EGM2)- *E. granulosus s.l.*: *19* labs (100%) out of 19 obtained a positive evaluation

Sample **3** (EGM3)- *Taenia crassiceps* (negative): 19 labs (100%) out of 19 obtained a positive evaluation

RESULTS

Laboratory code	N° of samples correctly identified	N° of samples NOT correctly identified	Final evaluation
Ed1	3	0	Positive
Ed2	3	0	Positive
Ed3	3	0	Positive
Ed4	3	0	Positive
Ed5	3	0	Positive
Ed6	3	0	Positive
Ed7	3	0	Positive
Ed8	3	0	Positive
Ed9	3	0	Positive
Ed10	3	0	Positive
Ed11	3	0	Positive
Ed12	3	0	Positive
Ed13	3	0	Positive
Ed14	3	0	Positive
Ed15	3	0	Positive
Ed16	3	0	Positive
Ed17	3	0	Positive
Ed18	3	0	Positive
Ed19	3	0	Positive

Conclusions

The experience derived from the fifth PT carried out in 2023 on the molecular detection of *Echinococcus* spp. showed that the personnel of NRLs are skilled to detect this parasite in a qualitative test

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

THANKS FOR YOUR ATTENTION