European Union Reference Laboratory for Parasites
Unit of Foodborne and Neglected Parasitic Diseases
Department of Infectious Diseases
ISTITUTO SUPERIORE DI SANITÀ

7th PROFICIENCY TESTING

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

XIX Workshop of National Reference Laboratories for Parasites
6th and 7th November 2024
Istituto Superiore di Sanità

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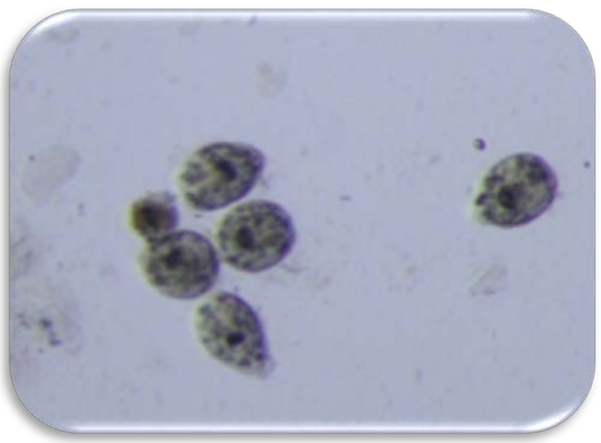
WHO Collaborating Centre for the Epidemiology, Detection and Control of Cystic and Alveolar Echinococcosis;
ISTITUTO SUPERIORE DI SANITÀ (Rome, Italy)

PT-08 Molecular identification of Echinococcus at the species level-2024

Due date to submit results: April 12, 2024
Individual report sent to participants within: April 30, 2024
Final report available on EURLP website from: May 31, 2024

Aim of the PT

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



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PT timing 2024

February 21st

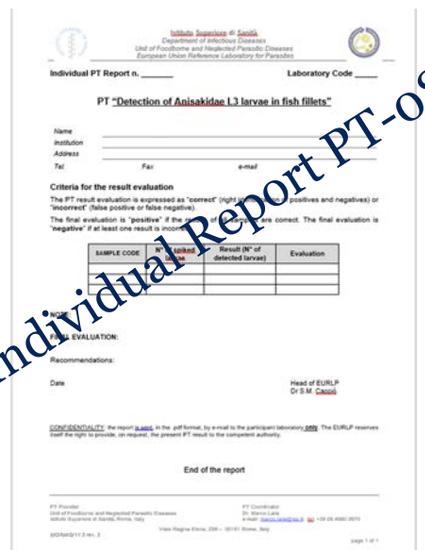
PTs REQUEST FORM 2024

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

March 11th

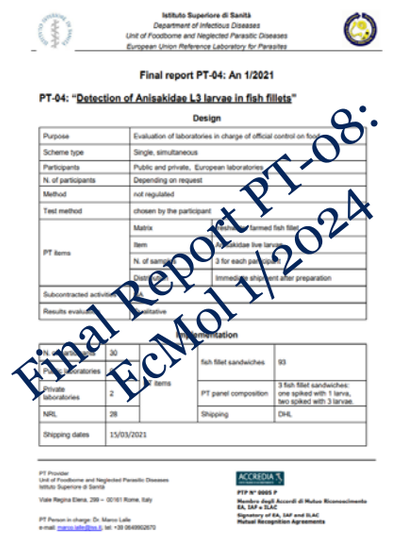
April 12th

April 30th



Individual Report PT-08

May 31st



Final Report PT-08: EcMol1/2024

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Preparation of samples PT08-2024

The PT has been organized following the NRL request (2023)

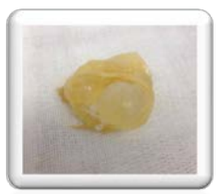
The panel consists of 3 tubes:

each tube containing DNA extracted from canine faecal stool **spiked**

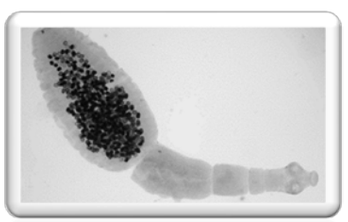
with

- *E. granulosus s.s.* cyst
- *E. multilocularis* worms
- DNA extracted from *Trichinella spp*. larvae

E. granulosus cysts collected from human hosts



E. multilocularis worms collected from foxes



The negative control was DNA extracted from *Trichinella spp.* larvae

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Detection method PT08-2024

The samples were identified at species level through multiplex PCR

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

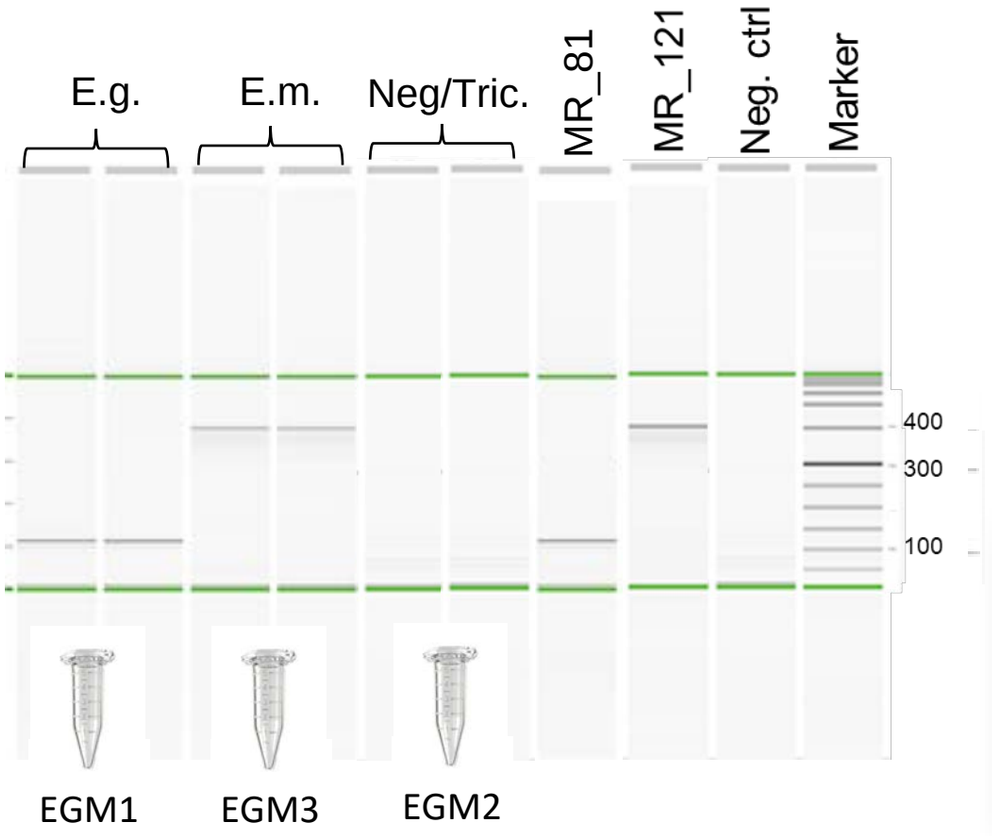
E.m. = *E. multilocularis* (395 bp)

Negative = *Trichinella*

EURLP-Reference Material

MR_81 = *E. granulosus* (G3)

MR_121= *E. multilocularis*



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

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Detection method PT08-2024

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

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Evaluation criteria

The PT evaluation is <u>qualitative</u> and no statistical analysis of the results are applied

Lab code	Expected	Observed	Result (correct/incorrect)	Evaluation (positive/negative)
EGM X	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. multilocularis*	correct correct correct	Positive
EGM X	*E. granulosus* negative *E. multilocularis*	*E. granulosus* *E. granulosus* *E. multilocularis*	correct incorrect correct	Negative
EGM X	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. granulosus*	correct correct incorrect	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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Partecipants

Austria	Austria NRLP, Austrian agency for health and food safety
Belgium	Belgium NRLP, Institute of Tropical Medicine
Denmark	Denmark NRLP, Statens Serum Institut, laboratory of parasitology, SSI
Estonia	Estonian NRLP, Animal Health, Veterinary and Food Laboratory
Finland	Finland NRLP, Oulu, Finnish Food Authority, Ruokavirasto (ex Evira)
France	France NRL Echinococcus, ANSES, LRFS Nancy
Germany	Germany NRL Echinococcus, Friedrich-Loeffler-Institut fur Epidemiologie
Iceland	Iceland, Institute for Experimental Pathology at Keldur
Ireland	Ireland NRLP, Parasit section, Bact/Paras Division, Backweston Campus, Celbridge Kildare
Latvia	Latvia NRLP, Institute of food safety, animal health and environment, BIOR
Norway	Norway NRLP, Norwegian Veterinary Institute
Poland	Poland NRLP, National Veterinary Research Institute , Department of Parasitology and Invasive Diseases
Portugal	Portugal NRLP, Instituto nacional de investigacao agraria e veterinaria
Republic of North Macedonia	Republic of North Macedonia, Faculty of Veterinary Medicine, Skopje
Romania	Romania NRLP, Institute for Diagnosis and Animal Health GMO and Molecular Biology Unit
Slovenia	Slovenian NRLP, University of Ljubljana, Veterinary Faculty
Spain	Spain NRLP, Laboratorio Central de Sanidad Animal
Switzerland	Switzerland, Institute of Parasitology University of Bern
The Netherlands	The Netherlands NRLP, National Institute for Public Health and the Evironment (RIVM)
United Kingdom	UK NRL for Trichinella and Echinococcus, Animal and Plant Health Agency, York (APHA)

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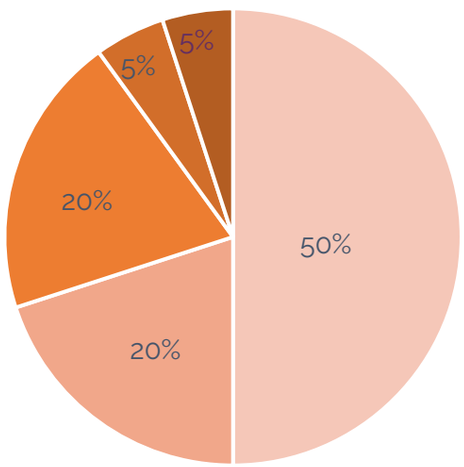
Detection method applied

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

Method applied	N. labs
Trachsel *et al*, 2007 (multiplex PCR)	10
Trachsel *et al* 2007 + other methods	4
EURLP method (MI-15) + sequencing or + Real Time PCR (Øines et al, 2014)	4
Geysen *et al*, 2007 (PCR RFLP) + sequencing	1
In house method (PCR RFLP)	1



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Results

Participants N=20

Participants passed N=17

Participants failed N=3

Sample 1 (*E. granulosus s. l.*): 19 labs (95%) out of 20 obtained a positive evaluation

Sample 2 (negative): 19 labs (95%) out of 20 obtained a positive evaluation

Sample 3 (*E. multilocularis*): 18 labs (90%) out of 20 obtained a positive evaluation

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Results

Lab Code	Expected	Observed	Result (correct/incorrect)	Evaluation (positive/negative)
EGM1 EGM2 EGM3	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. multilocularis*	correct correct correct	positive
EGM4 EGM5 EGM6	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. multilocularis*	correct correct correct	positive
EGM7 EGM8 EGM9	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. multilocularis*	correct correct correct	positive
EGM10 EGM11 EGM12	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. multilocularis*	correct correct correct	positive
EGM13 EGM14 EGM15	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. multilocularis*	correct correct correct	positive
EGM16 EGM17 EGM18	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. multilocularis*	correct correct correct	positive
EGM19 EGM20 EGM21	*E. granulosus* negative *E. multilocularis*	*E. granulosus* *E. granulosus* *E. multilocularis*	correct incorrect correct	negative
EGM22 EGM23 EGM24	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. multilocularis*	correct correct correct	positive
EGM25 EGM26 EGM27	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. multilocularis*	correct correct correct	positive
EGM28 EGM29 EGM30	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. multilocularis*	correct correct correct	positive

Lab Code	Expected	Observed	Result (correct/incorrect)	Evaluation (positive/negative)
EGM31 EGM32 EGM33	*E. granulosus* negative *E. multilocularis*	negative negative -	incorrect correct incorrect	negative
EGM34 EGM35 EGM36	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. multilocularis*	correct correct correct	positive
EGM37 EGM38 EGM39	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. granulosus*	correct correct incorrect	negative
EGM40 EGM41 EGM42	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. multilocularis*	correct correct correct	positive
EGM43 EGM44 EGM45	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. multilocularis*	correct correct correct	positive
EGM46 EGM47 EGM48	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. multilocularis*	correct correct correct	positive
EGM49 EGM50 EGM51	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. multilocularis*	correct correct correct	positive
EGM52 EGM53 EGM54	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. multilocularis*	correct correct correct	positive
EGM55 EGM56 EGM57	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. multilocularis*	correct correct correct	positive
EGM58 EGM59 EGM60	*E. granulosus* negative *E. multilocularis*	*E. granulosus* negative *E. multilocularis*	correct correct correct	positive

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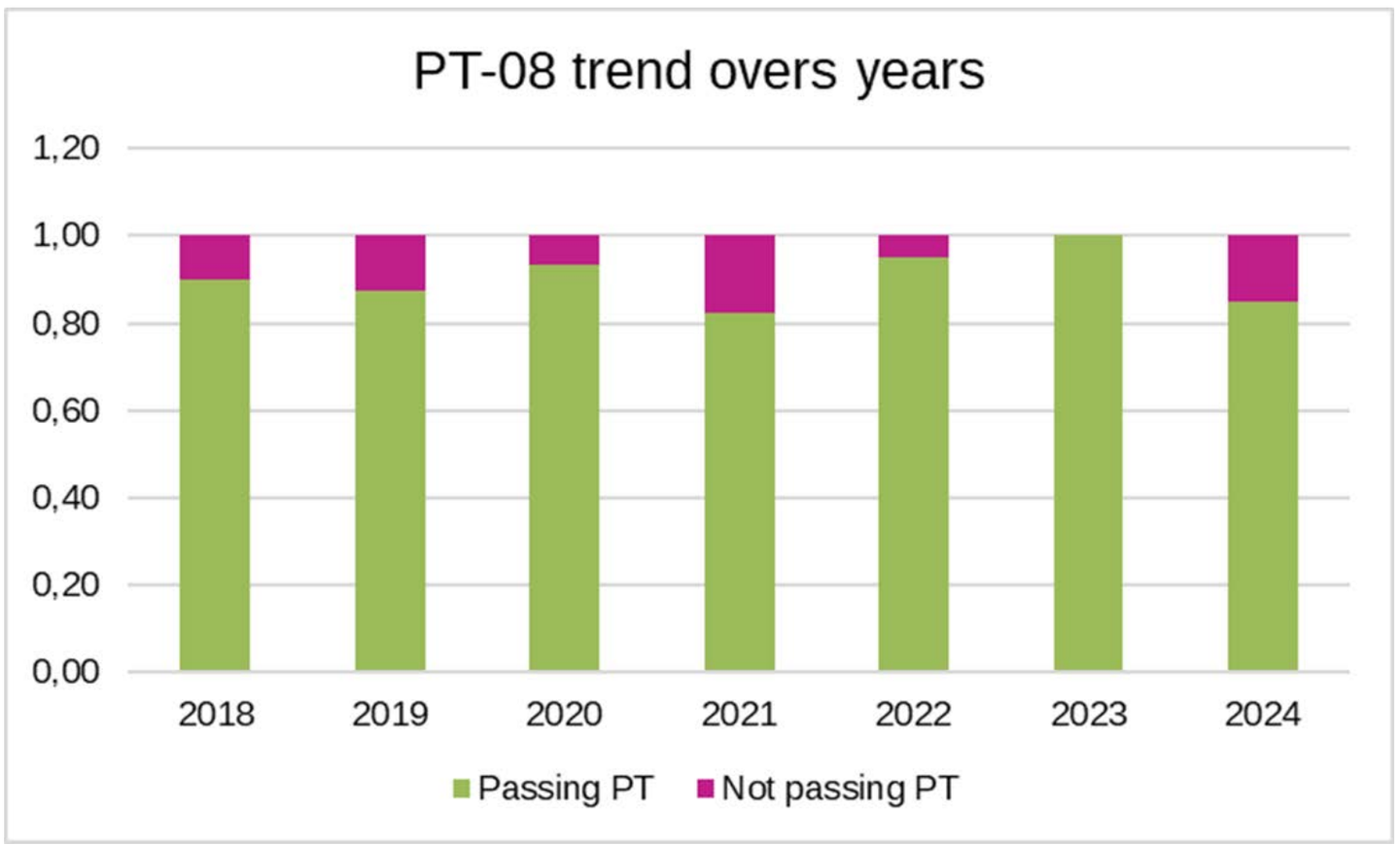
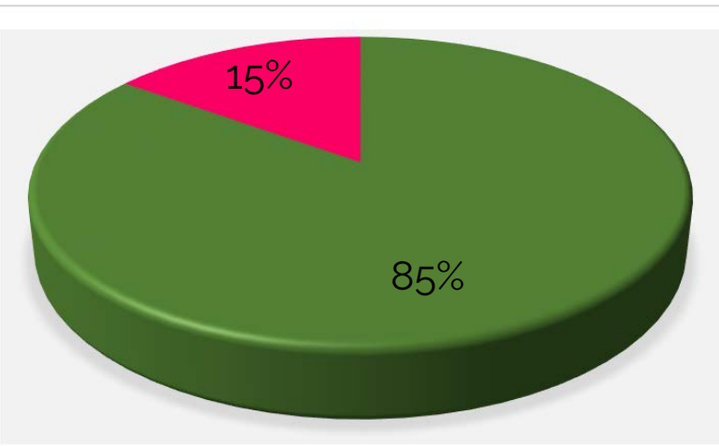
Results



2024



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Conclusions

85% laboratories passed the Proviciency Testing 2024

95% of participants correctly identified the sample with DNA of *E. granulosus s.l.*

90% succeeded in the identification of the sample with DNA of *E. multilocularis*

95% detected the negative sample

Recommendations

- to optimize multiplex PCR settings to avoid unspecific amplifications and to sequence amplicons to confirm the identification
- to select the most suitable protocol for the identification of the two species covered by the Proficiency Testing

Thanks for your attention

ACKNOWLEDGMENTS

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EURLP