



13th PROFICIENCY TESTING on:

“Detection of *Echinococcus* spp. worms in the intestinal mucosa of the definitive host”



Azzurra Santoro, Simona Cherchi, Maria Interisano,
Federica Santolamazza, Alessia Possenti, Adriano Casulli



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 on *Echinococcus* spp. in the intestinal mucosa is accredited in a quality system according to ISO 17043 standard

Aim: detection of 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* sp.

TIMING:

PTs announced: 25/01/2022

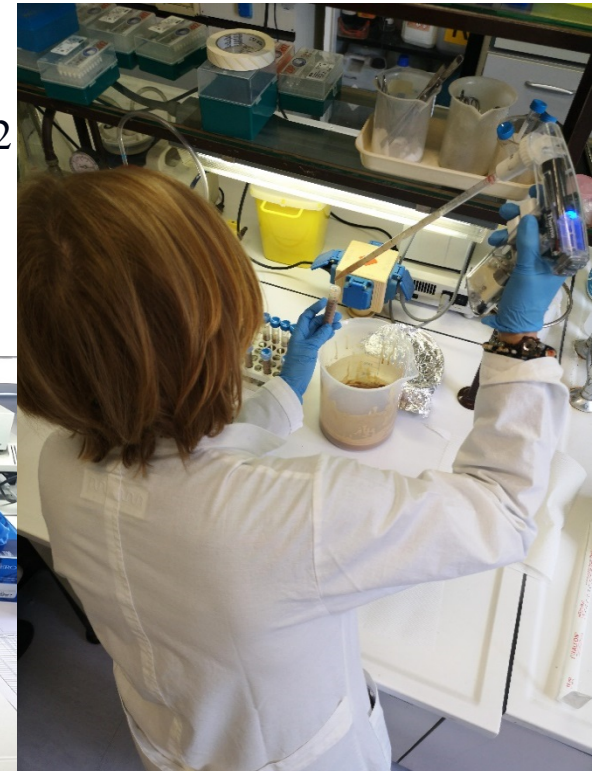
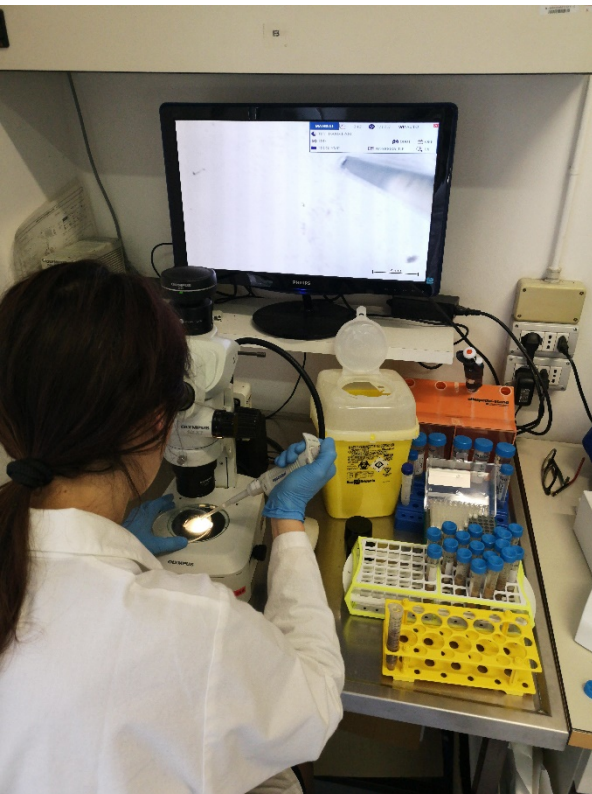
Deadline participation form: 25/02/2022

PTs dispatched to participants: 14/03/2022

Reporting deadline: 25/03/2022

Individual PT reports: 11/04/2022

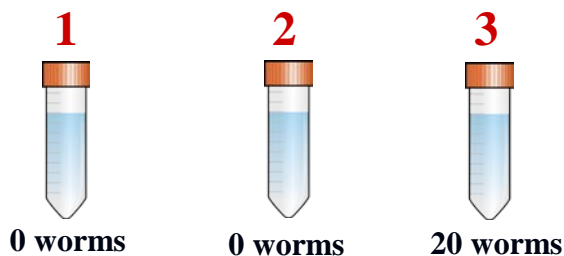
Final report online: 31/05/2022



Preparation of samples

- Fox **intestines** were collected and stored **frozen** at -80°C for one week.
- ***E. multilocularis*** infections were excluded.
- The **mucosa** of the small intestine of foxes was collected, cleaned and sent to ISS.
- Mucosa was **homogenised** (with **70% ethanol**; ratio 2:1), aliquoted, and spiked (double check).

PT panel:



We are very grateful to:

JACEK KARAMON (NRL Poland), **PAVLO MAKSIMOV** (NRL Germany), **GERALD UMHANG** (NRL France) and **ANTTI OKSANEN** (NRL Finland) for having provided worms of *Em* and intestinal mucosa.

Participants (N=28)



Albania, Institute of Public Health

Austria NRLP, Austrian agency for health and food safety

Belgium NRLP, Institute of Tropical Medicine

Bulgaria NRLP, National Diagnostic and Research Veterinary Institute

Cyprus NRLP, Veterinary Services

Czech Republic NRLP, State Veterinary Institute

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 für Epidemiologie

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, Backweston Campus, Celbridge Kildare

Italy NRL Echinococcus, IZS Istituto Zooprofilattico Sperimentale della Sardegna

Latvia NRLP, Institute of food safety, animal health and environment, BIOR

Lithuanian NRLP, National Food and Veterinary Risk Assessment Institute

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

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, SVA

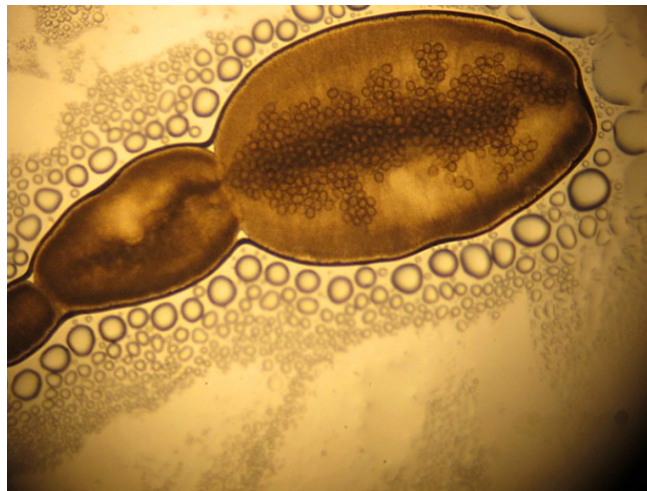
UK NRL for Trichinella and Echinococcus, Animal and Plant Health Agency, York

UK (Northern Ireland) AgriFood and Busciences Institute (AFBI), Coneywarren, Omagh

Criteria for the **qualitative** evaluation

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

...OR **INCORRECT** (false positive or false negative results), irrespective of the number of worms in the samples.
- 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.



SUMMARY of RESULTS (**Qualitative** evaluation)

Number of participant laboratories submitting results	28
Number of participants that passed the PT	14
Number of participants that failed the PT	14



Photo courtesy of Andrew Hemphill

Laboratory code	N° of items correctly identified	N° of items NOT correctly identified	Kind of error	Final evaluation
Em1	2	1	False positive	NEGATIVE
Em2	3	0		POSITIVE
Em3	3	0		POSITIVE
Em4	2	1	False positive	NEGATIVE
Em5	3	0		POSITIVE
Em6	2	1	False positive	NEGATIVE
Em7	3	0		POSITIVE
Em8	3	0		POSITIVE
Em9	3	0		POSITIVE
Em10	3	0		POSITIVE
Em11	3	0		POSITIVE
Em12	3	0		POSITIVE
Em13	1	2	False positive	NEGATIVE
Em14	3	0		POSITIVE
Em15	2	1	False positive	NEGATIVE
Em16	1	2	False positive	NEGATIVE
Em17	2	1	False positive	NEGATIVE
Em18	3	0		POSITIVE
Em19	2	1	False positive	NEGATIVE
Em20	1	2	False positive	NEGATIVE
Em21	2	1	False positive	NEGATIVE
Em22	3	0		POSITIVE
Em23	2	1	False positive	NEGATIVE
Em24	2	1	False positive	NEGATIVE
Em25	2	1	False positive	NEGATIVE
Em26	2	1	False positive	NEGATIVE
Em27	3	0		POSITIVE
Em28	3	0		POSITIVE

RESULTS (Qualitative evaluation)

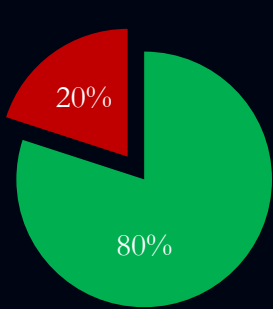


QUALITATIVE evaluation:

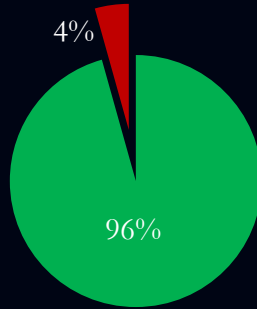
- **Sample 1** (negative sample): 21 laboratories (**75%**) out of 28 correctly identified the item.
- **Sample 2** (negative sample): 18 laboratories (**64%**) out of 28 correctly identified the item.
- **Sample 3** (20 worms): 28 laboratories (**100%**) out of 28 correctly identified the item.



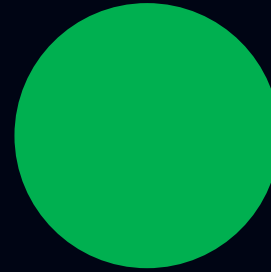
TREND: QUALITATIVE EVALUATION (2012-2022)



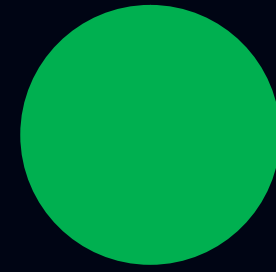
2012



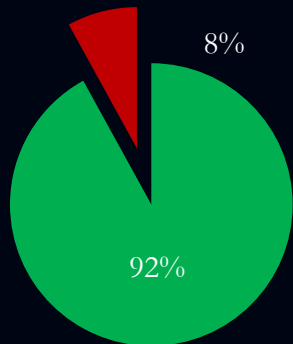
2013



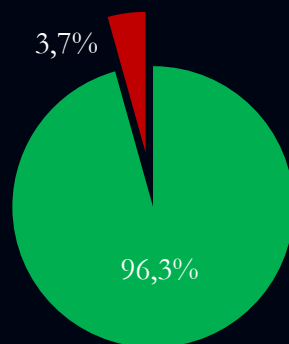
2014



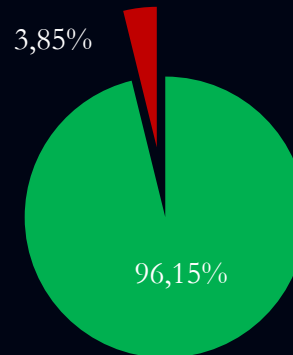
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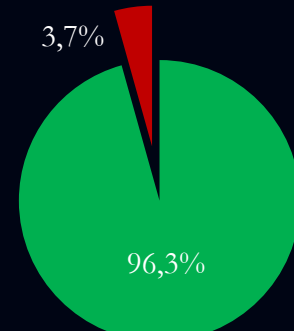
2016



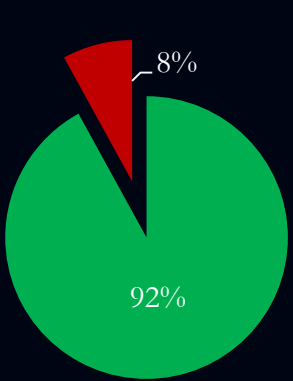
2017



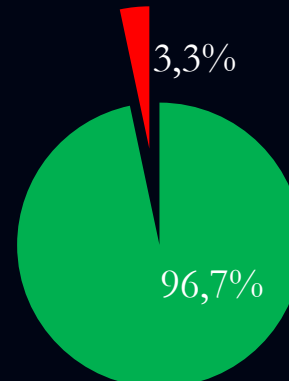
2018



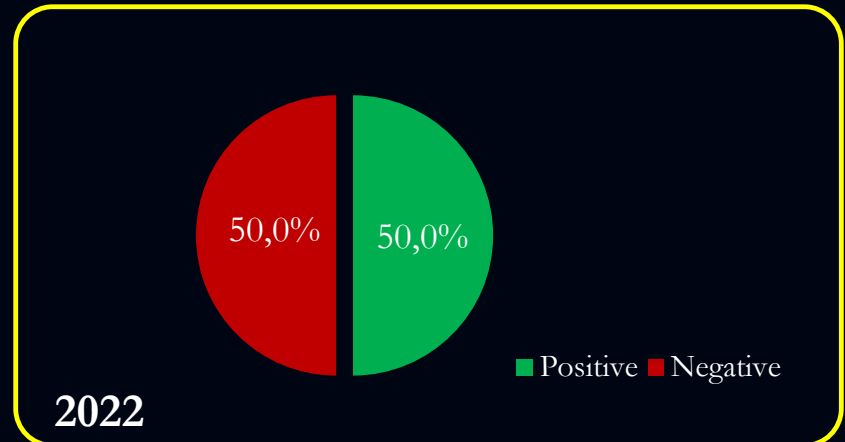
2019



2020



2021

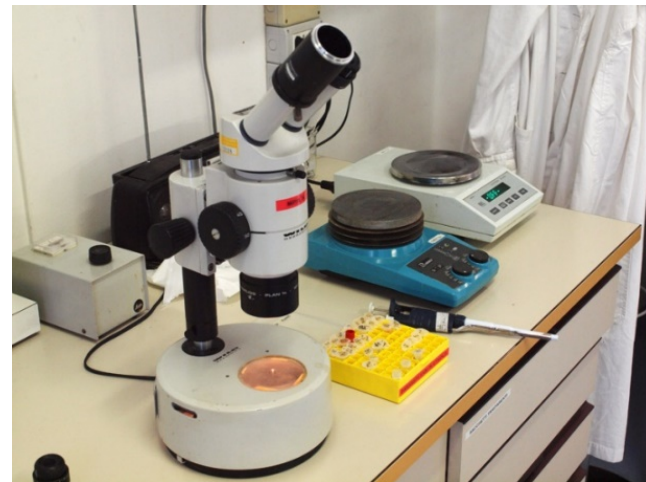


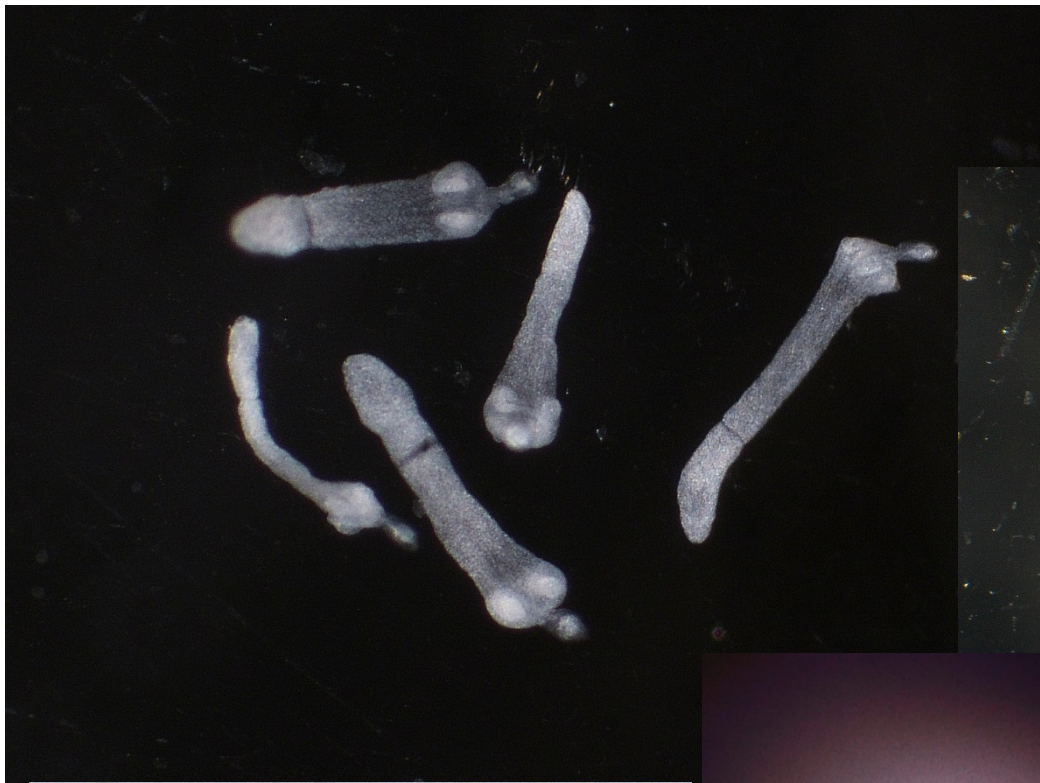
2022

Tricky worms??

- Compared to previous years, a significant number of laboratories mis-identified one or both the negative items as positive.
- Intestinal mucosa, provided for PT preparation, was collected from foxes tested as negative for *E. multilocularis* infections, from a member state who implements surveillance and reporting to maintain the status “*E. multilocularis* free”
- Several laboratories, both passing or not the PT, had actually noticed the presence of particular worms similar to but different from *Echinococcus* in the negative samples.

After the conclusion of the PT, many participants shared microscopic images of the suspicious worms with us...



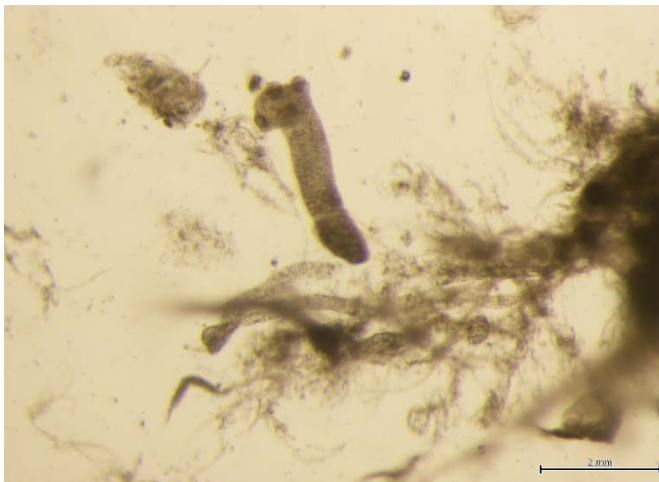






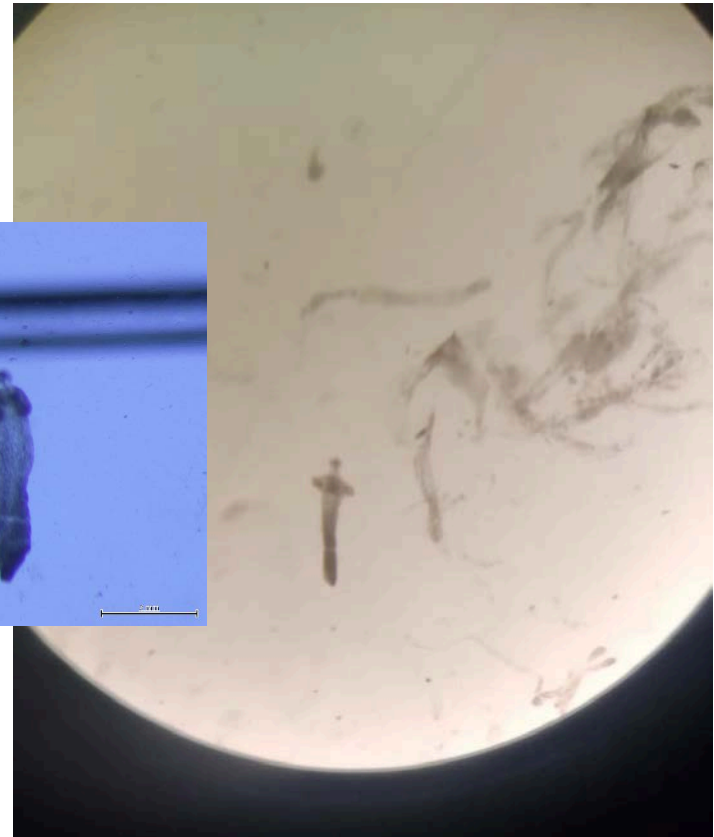
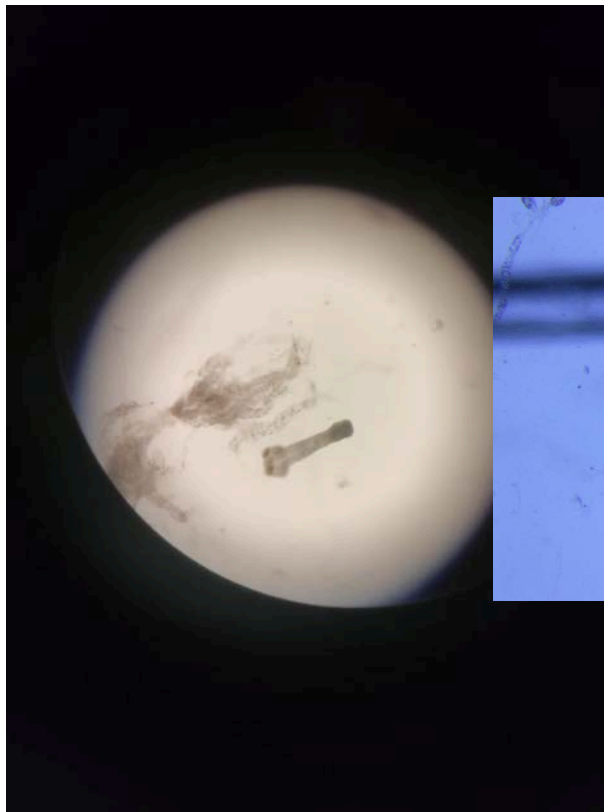
And then here at the EURLP

Pictures taken during the examination of the surplus PT panels
(stability check in April 2022)



And then here at the EURLP

Pictures taken during the examination of the surplus PT panels
(stability check in April 2022)



Echinococcus-like worms in carnivore mucosa?

Previous reporting?

Veterinary Parasitology: Regional Studies and Reports 22 (2020) 100470

Contents lists available at ScienceDirect

Veterinary Parasitology: Regional Studies and Reports

journal homepage: www.elsevier.com/locate/vprsr

ELSEVIER

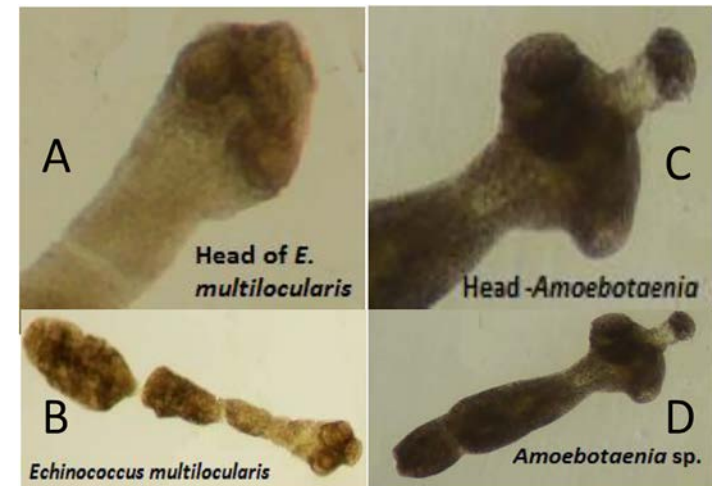
Short Communication

Echinococcus multilocularis in red foxes in North Belgium: Prevalence and trends in distribution

F. Jansen^{a,1,*}, M. Claes^{a,d,1}, E. Bakkers^a, A. Aryal^{a,e}, K.C. Madimba^{a,f}, S. Gabriël^{a,g}, V. Dermauw^a, A. Van Hul^a, M. Vervaeke^b, P. Dorny^{a,c}

- Cross-sectional survey in Flanders, Belgium
- Over 923 foxes examined, small cestodes detected in 38
- Of these, 12 and 7 identified as *Amoebotaenia* sp and mixed *Amoebotaenia/E. multilocularis* positive, respectively

“Morphological differentiation between *Echinococcus multilocularis* (A,B) and *Amoebotaenia* sp. (C,D) (...) (source: French National Reference Laboratory (NRL) for *Echinococcus* spp.)” from Jansen et al. 2020



Echinococcus-like worms in carnivore mucosa?

Previous reporting?

- *Amoebotaenia paradoxa*, Rudolphi 1802
- **Petavy and Deblock, 1980**: Helminthes du renard commun (*Vulpes vulpes*) dans la région du massif central (France). Ann Parasitol Hum Comp. 1980;55(4):379-91.
- **Deblock et al., 1988**: Helminthes intestinaux du Renard commun (*Vulpes vulpes*) dans le Massif central (France). Can. J. Zool. 66 : 1562 - 1569.
- **Petavy et al., 1990**: Epidémiologie de l'échinococcose alvéolaire en France: helminthes intestinaux du renard commun (*Vulpes vulpes*) en Haute-Savoie, Ann. Parasitol. Hum. Comp., 1990, 65 : n° 1, 22-27.

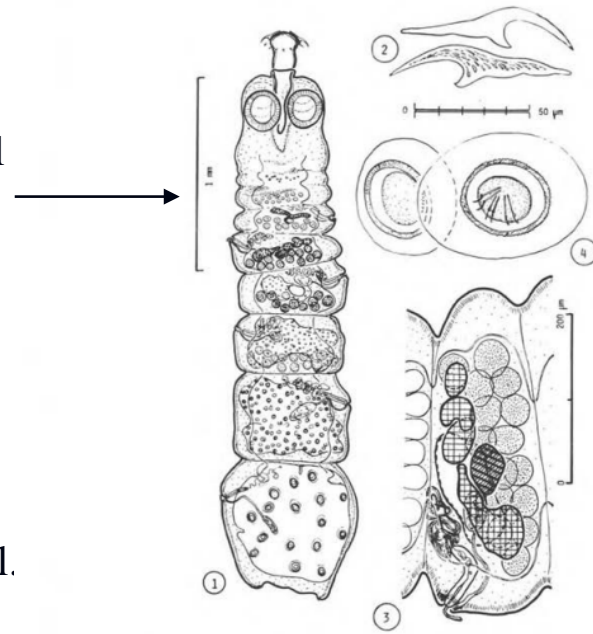


Fig. 7. *Amoebotaenia paradoxa*. 1. Cestode adulte complet. 2. Crochets du rostre. 3. Anneau mûr. 4. Œufs mûrs.


The French experience

- *A. paradoxa* parasite of birds (Charadriiformes)
- Intermediate host: earthworms
- Low prevalence in red foxes

Echinococcus-like worms in carnivore mucosa?

Previous reporting?

The French experience

 **Diagnose différentielle rapide**
pour *E. multilocularis*

Octobre 2016
Anses LRFSN
LNR *Echinococcus* spp.

agence nationale de sécurité sanitaire
alimentation, environnement, travail
Connaître, évaluer, protéger

A practical guide from ANSES for technicians performing SSCT



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

Echinococcus-like worms in carnivore mucosa?

Previous reporting?

The French experience

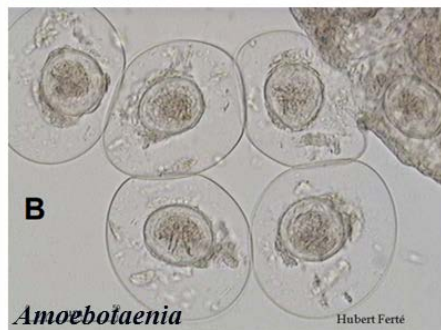
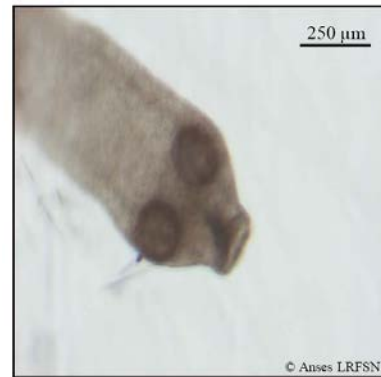
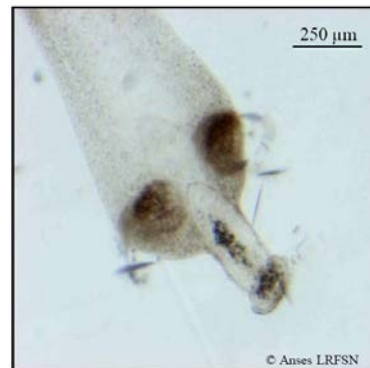


Figure 3 : Œufs in utero A - *Echinococcus multilocularis*
 B - Cestode de type "Amoebotaenia"

Photos by Hubert Fertè



Scolex d'*E. multilocularis*



Scolex de cestode de type *Amoebotaenia*

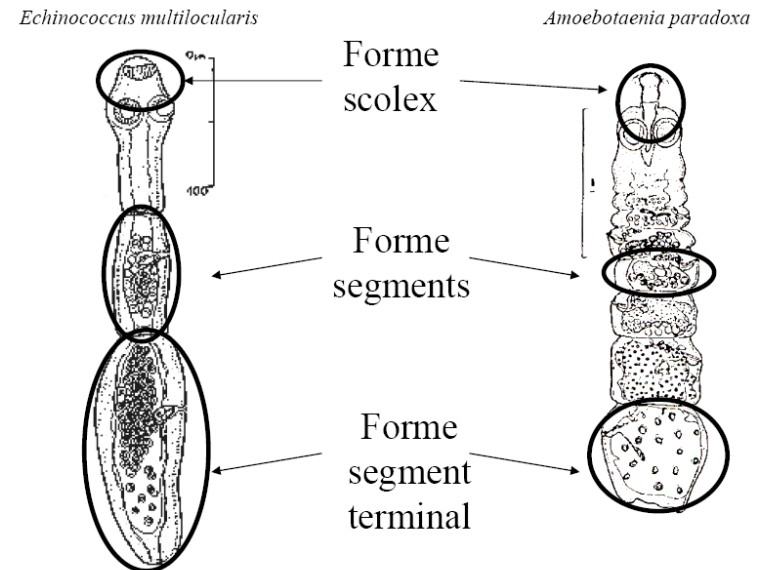


Schéma original d'*E. multilocularis* d'après Leuckart (1853) et schéma original d'*Amoebotaenia paradoxa* d'après Petavy et Deblock (1980).

Contact: Gérald UMHANG
gerald.umhang@anses.fr

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

Echinococcus-like worms in carnivore mucosa?

Previous reporting?

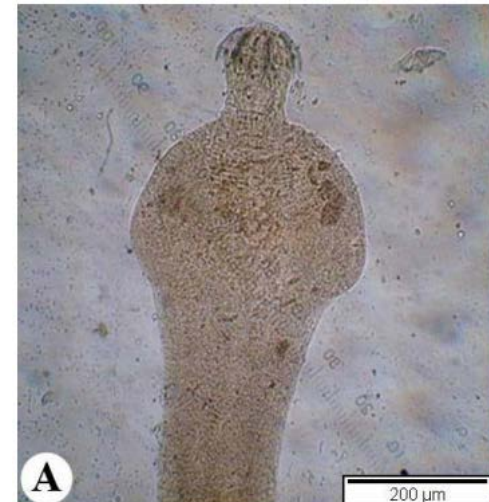
NO MOLECULAR CONFIRM

No *Amoebotaenia* spp sequences in GenBank (only a small ITS2 fragment of *A. cuneata*)

12S appears the suitable marker for future characterization

(suggested: semi-nested PCR (Geysen et al., 2007))

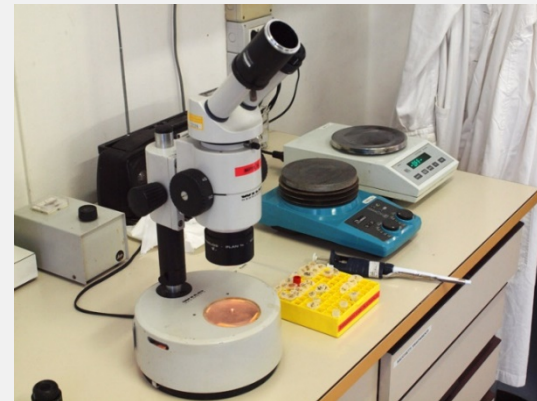
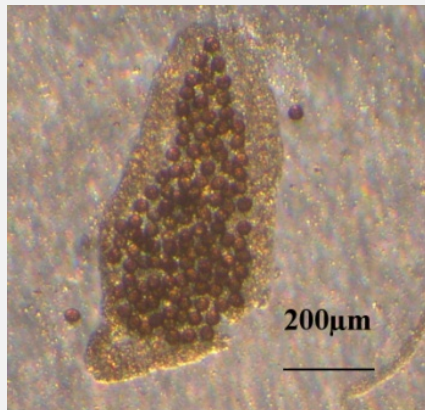
	nBLAST identity
<i>Drepanidotaenia lanceolata</i>	78%
<i>Mesocestoides corti</i>	77%
<i>Duplicibothrium</i> sp.	77%
<i>Potamostrongyloides</i> sp.	77%



Drepanidotaenia sp. from Rzayev et al. 2021

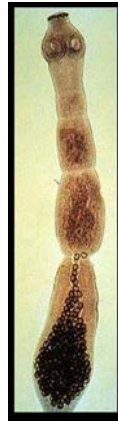
Outcomes from 2022....

- This year, most of the participants encountered a “new” cestode-like parasite that was misidentified as *E. multilocularis*, therefore resulting in a low performance by laboratories.
- This occasion called the attention to the importance of differential diagnosis with *E. multilocularis* when examining fox intestinal content.
- It would be a great opportunity, for the network of NRL, to cooperate collecting epidemiological and molecular data on this parasite, and produce a molecular algorithm to help differential diagnosis in future.



ACKNOWLEDGMENTS:

- Simona Cherchi (“worming” the samples / packing PTs)
- Maria Interisano (mixing the mucosa / packing PTs)
- Alessia Possenti (supervising Echino_PTs & quality system)
- Federica Santolamazza (preparation molecular & SCT PTs / data managing / packing PTs)



Thanks for the attention!