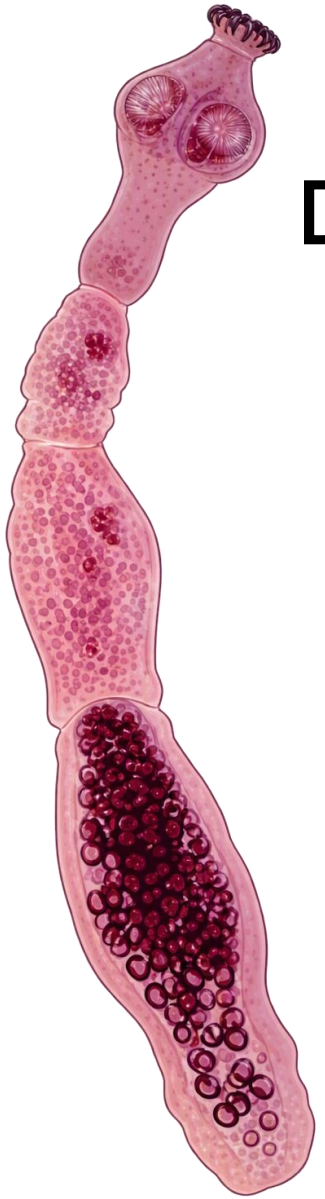


PT-05

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

Azzurra SANTORO, Francesco CELANI, Antonio DI GRAZIA, Federica SANTOLAMAZZA, Irene TARTARELLI

Workshop of the National Reference Laboratories for Parasites,
Rome, Italy, 28-29 October 2026

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: PT panel consisted of three tubes containing homogenised intestinal mucosa, either spiked or not with *Echinococcus* worms.

CALL FOR PARTICIPING	Jan 27th
DEADLINE FOR SUBSCRIPTION	Feb 20th
PT SENDING	Mar 16th
DEADLINE FOR SUBMITTING RESULTS	Mar 23rd
FINAL REPORT PUBLISHED ON THE EURLP WEBPAGE	May 29th

No more individual reports

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.

✅ Positive sample + worms detected = Correct

✅ Negative sample + no worms detected = Correct

❌ False positive = Incorrect

❌ False negative = Incorrect



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.

Participant code	Applied method	PT item code	Result	Assigned value	Outcome	Final evaluation
Emu1	SCT	Emu01	8	20	correct	Positive
		Emu02	0	0	correct	
		Emu03	0	0	correct	
Emu2	In house method	Emu04	10	20	correct	Positive
		Emu05	0	0	correct	
		Emu06	0	0	correct	

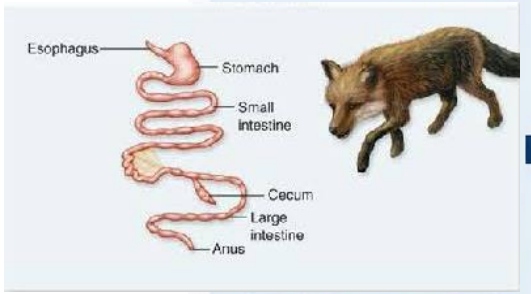
Albania: Institute of Public Health
Austria: Austrian Agency for Health and Food Safety (AGES)
Belgium: Institute of Tropical Medicine, Department of Animal Health
Croatia: Croatian Veterinary Institute
Cyprus: Laboratory for the Control of Food of Animal Origin
Czech Republic: State Veterinary Institute Olomouc
Denmark: Statens Serum Institut, Laboratory of Parasitology
Estonia: National Centre for Laboratory Research and Risk Assessment
Finland: Finnish Food Authority
France: ANSES LRFSN, National Reference Laboratory for *Echinococcus* spp.
Germany: Friedrich-Loeffler-Institut
Greece: Athens Center of Veterinary Institutes
Hungary: National Food Chain Safety Office
Iceland: Keldur, Institute for Experimental Pathology
Ireland: Department of Agriculture, Food and the Marine (DAFM Laboratories)
Italy: Istituto Zooprofilattico Sperimentale della Sardegna
Latvia: Institute of Food Safety, Animal Health and Environment "BIOR"
Lithuania: National Food and Veterinary Risk Assessment Institute
N Ireland: Department of Agriculture, Food and the Marine (DAFM Laboratories)
Norway: Norwegian Veterinary Institute
Poland: National Veterinary Research Institute PiWet
Portugal: Instituto Nacional de Investigação Agrária e Veterinária (INIAV)
Republic of North Macedonia: University "Ss. Cyril & Methodius" Skopje
Romania: Institute For Diagnosis and Animal Health
Serbia: Institute for Laboratory Diagnostic INEP
Slovak Republic: State Veterinary and Food Institute Bratislava
Slovenia: University of Ljubljana, Veterinary Faculty
Spain: Laboratorio Central de Sanidad Animal de Santa Fe
Sweden: Swedish Veterinary Agency (SVA)
Switzerland: Institute of Parasitology, Vet-Suisse Faculty, University of Bern
The Netherlands: National Institute of Public Health and the Environment (RIVM)
UK: Animal and Plant Health Agency (APHA)

32 PARTICIPATING COUNTRIES



PT-05 preparation workflow

1 Fox intestine



- Fox intestines collected by providers
- Stored frozen at -80°C for 1 week

2 Mucosa collection



- Small intestinal mucosa collected and cleaned
- Sent to ISS

3 Homogenisation



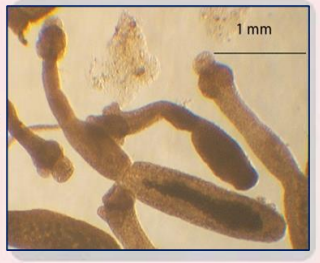
- Mucosa homogenised in 70% ethanol (2:1, w/v)

4 Aliquoting



- Homogenate aliquoted into tubes

5 Spiking with worms



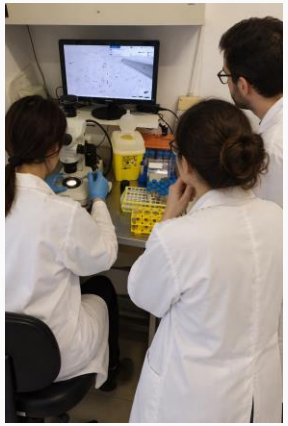
- Worms previously deactivated at -80°C (by providers)
- Aliquots spiked or not spiked (double check)

6 Distribution to laboratories



- Three tubes sent to each participating laboratory

Faecal content is analysed by qPCR to exclude *E. multilocularis* infection.



PT panel (3 tubes per laboratory)



Item 1
20 worms
(positive)

Item 2
0 worms
(negative)

Item 3
0 worms
(negative)

Acknowledgements

PT material kindly provided by:

Finnish Food Authority
(Ruokavirasto, Finland)

and

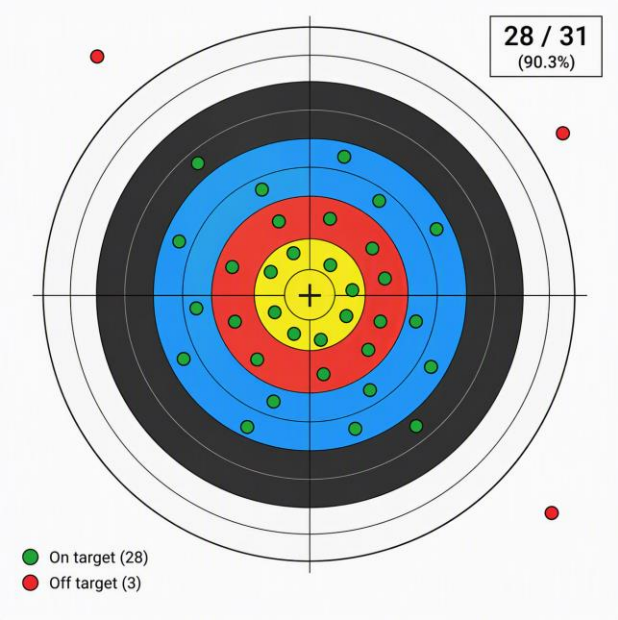
Croatian Veterinary Institute
(Croatia)

SUMMARY OF RESULTS

Number of participant laboratories submitting results	**31/**32 (96,87%)
Number of participants that passed the PT	**14**/31 (45.16%)
Number of participants that failed the PT	**17**/31 (54,84%)

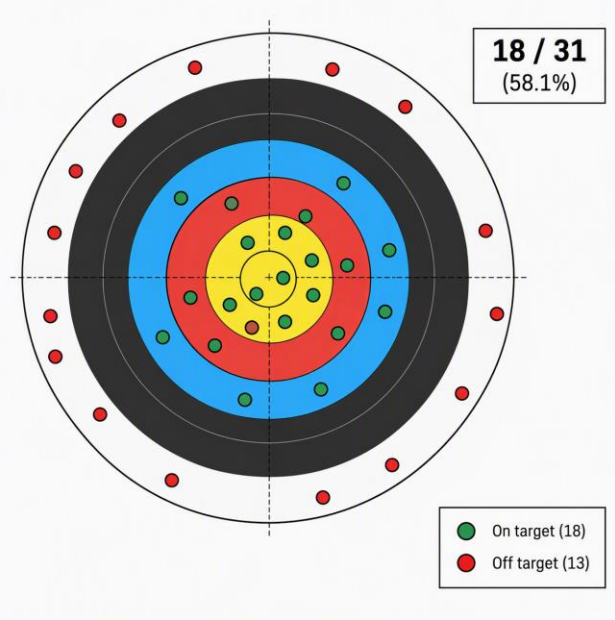
1° ITEM (positive, 20 worms)

Mean of detection: 9,3



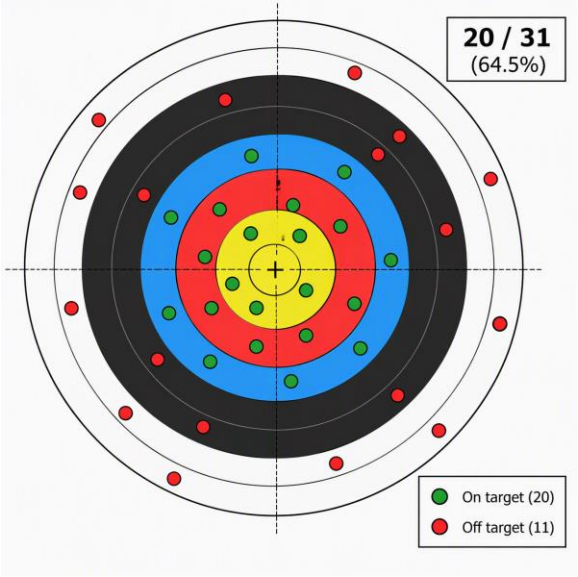
3 FALSE negative results

2° ITEM (negative, 0 worms)



13 FALSE positive results

3° ITEM (negative, 0 worms)



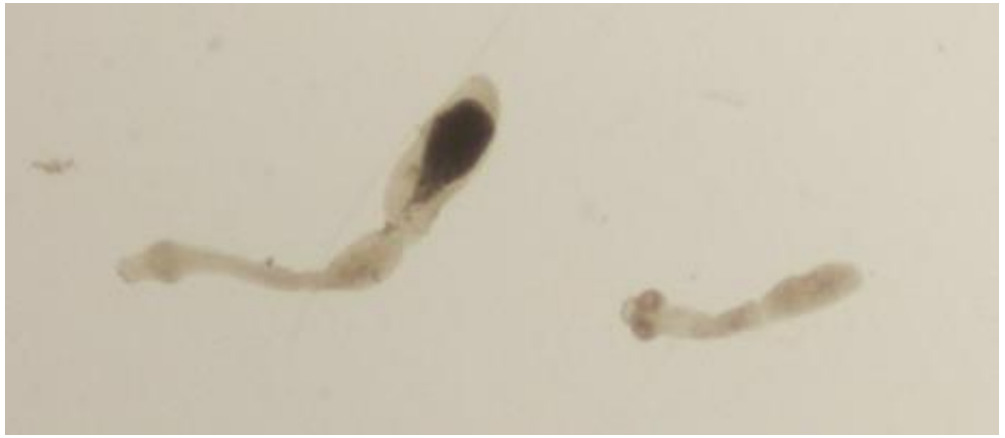
11 FALSE positive results

RESULTS: METHODOLOGY

- Most laboratories used the Sedimentation and Counting Tecnique (**SCT**) with no relevant modification.
- One lab directly diluted the samples drop by drop in water under the binocular microscope.
- One lab used water insetad of NaCl 0,9% and examined both sediment and supernatant.

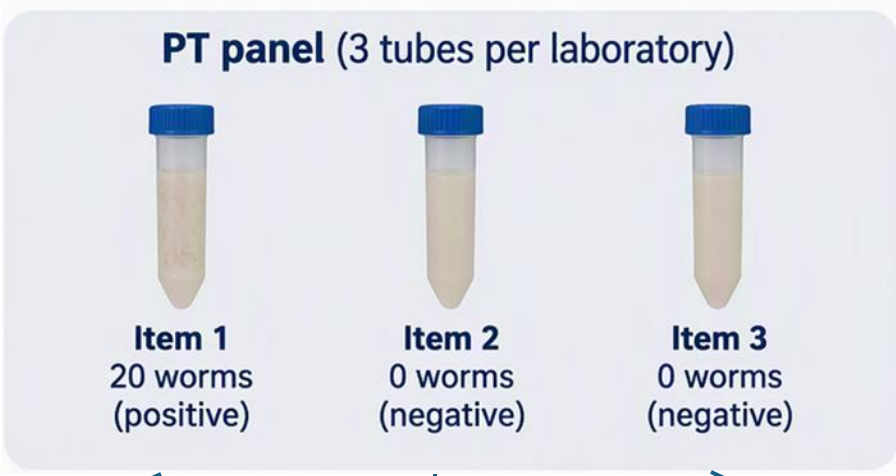
- Three labs (3/31) poured the samples through a sieve
  - -180 uM
  - -150 uM
  - - 50 uM





No evident correlation between methodology and outcome.

RESULTS: OBSERVATIONS from PARTICIPANTS



- 6: *Amoebotaenia*-like worms
- 1: not *Echinococcus* worms
- 1: PCR (confirming positivity)

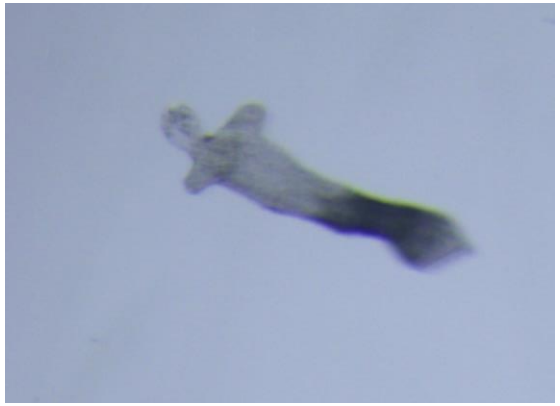
- 12: *Amoebotaenia*-like worms
- 1: not *Echinococcus* worms
- 1: PCR (confirming negativity)
- 1: other elminths (*Uncinaria*)

- 12: *Amoebotaenia*-like worms
- 1: not *Echinococcus* worms
- 1: PCR (confirming negativity)

Observation of *Amoebotaenia*-like cestodes was correlated with correct identification

ROOT CAUSE ANALYSIS OF DISCORDANT RESULTS

The intestinal mucosa used for PT-05 2026 was **naturally infected** with cestodes **morphologically similar**, but **distinct** from *Echinococcus* spp., here referred to as *Amoebotaenia*-like cestodes.



Investigation of discordant results was conducted through discussions with the participating laboratories (FOLLOW-UP form).

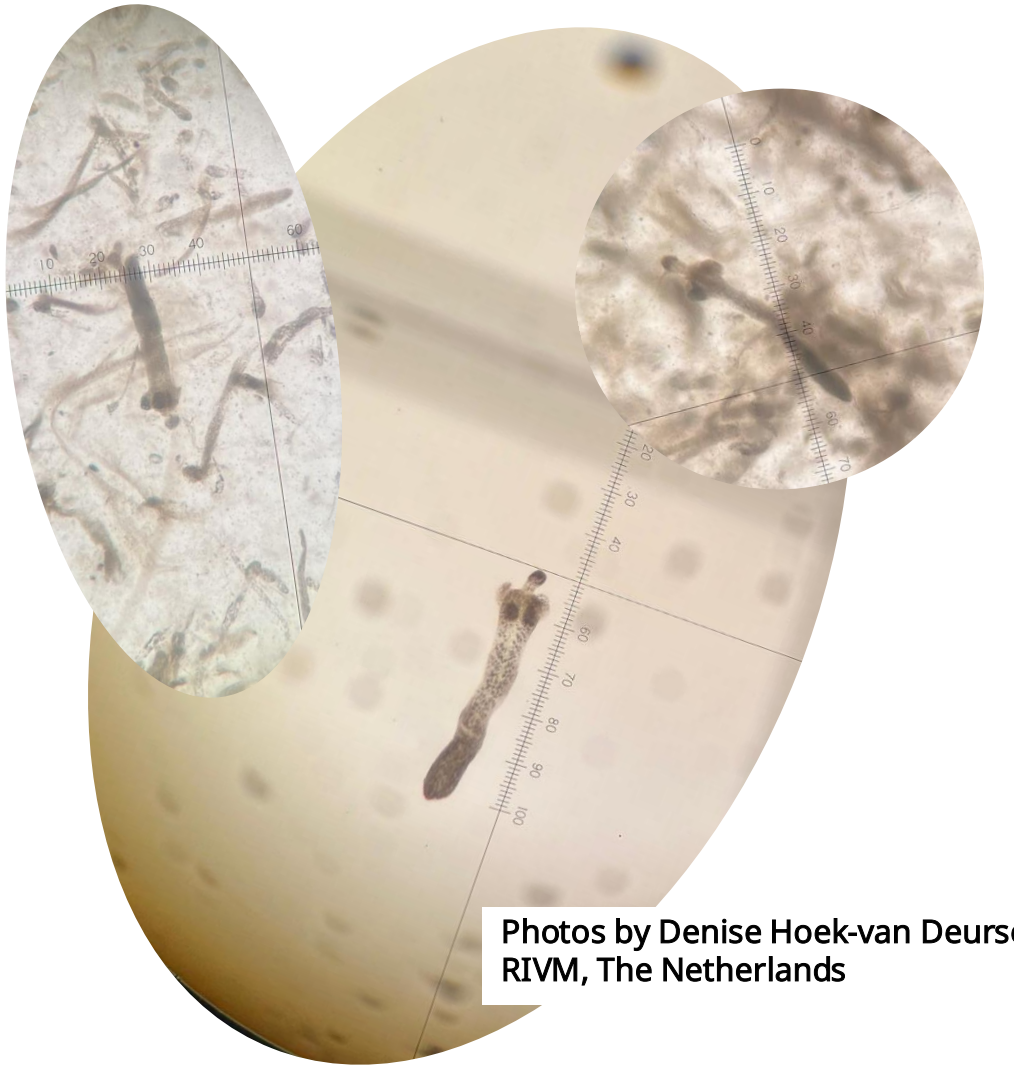
- The false-negative result was attributed to a possible partial loss of sediment during sample processing and examination.
- The false-positive results were mainly attributed to:

-limited experience in *Echinococcus* identification;

-lack of awareness of non-*Echinococcus* cestodes that may closely resemble *Echinococcus* specimens.

AMOEBOTAENIA (?) -LIKE WORMS, AGAIN

- Similar occurrence in **2025**-PT05.



Photos by Denise Hoek-van Deursen
RIVM, The Netherlands



Photo by Famke Jansen
ITM, Belgium

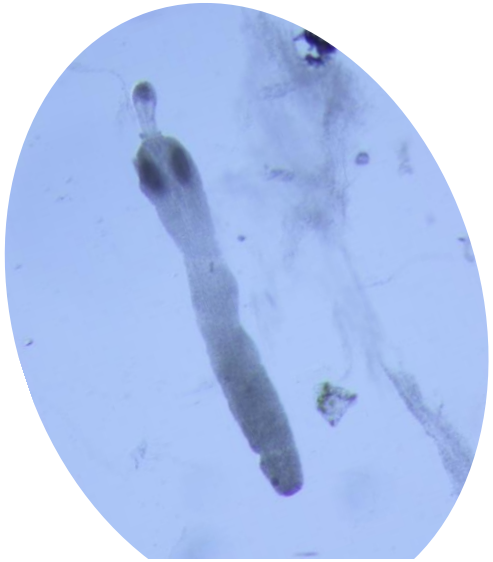
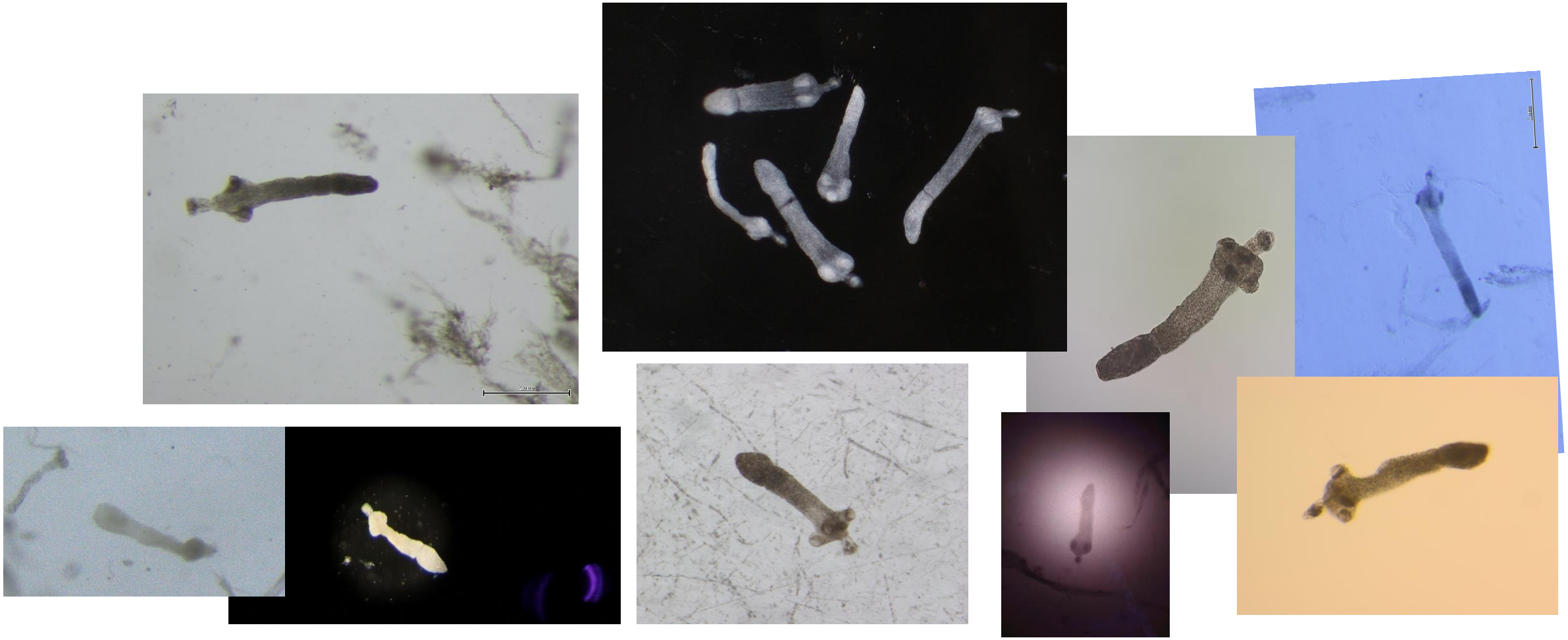


Photo by Federica Santolamazza
EURL-P, Italy

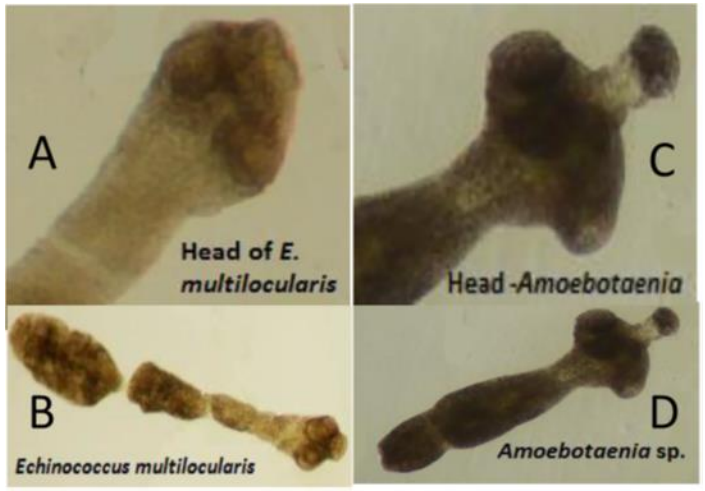
AMOEBOTAENIA (?) -LIKE WORMS, AGAIN

- Similar occurrence in **2022**-PT05.



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

AMOEBOTAENIA (?) -LIKE WORMS, AGAIN







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

Feature	*Echinococcus multilocularis*	*Amoebotaenia*-like cestode
Body form	Slender strobila	Compact strobila
Rostellum	Less prominent	More prominent and protruding
Rostellar hooks	Blade, base and handle clearly distinguishable	Hooks less curved, base absent or poorly developed
Terminal proglottid	Elongated	Short and broad
Uterus in gravid segment	Well visible, centrally located and filled with eggs	Less evident

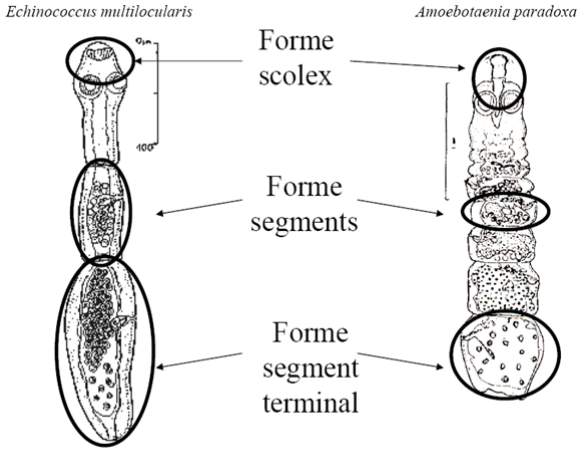
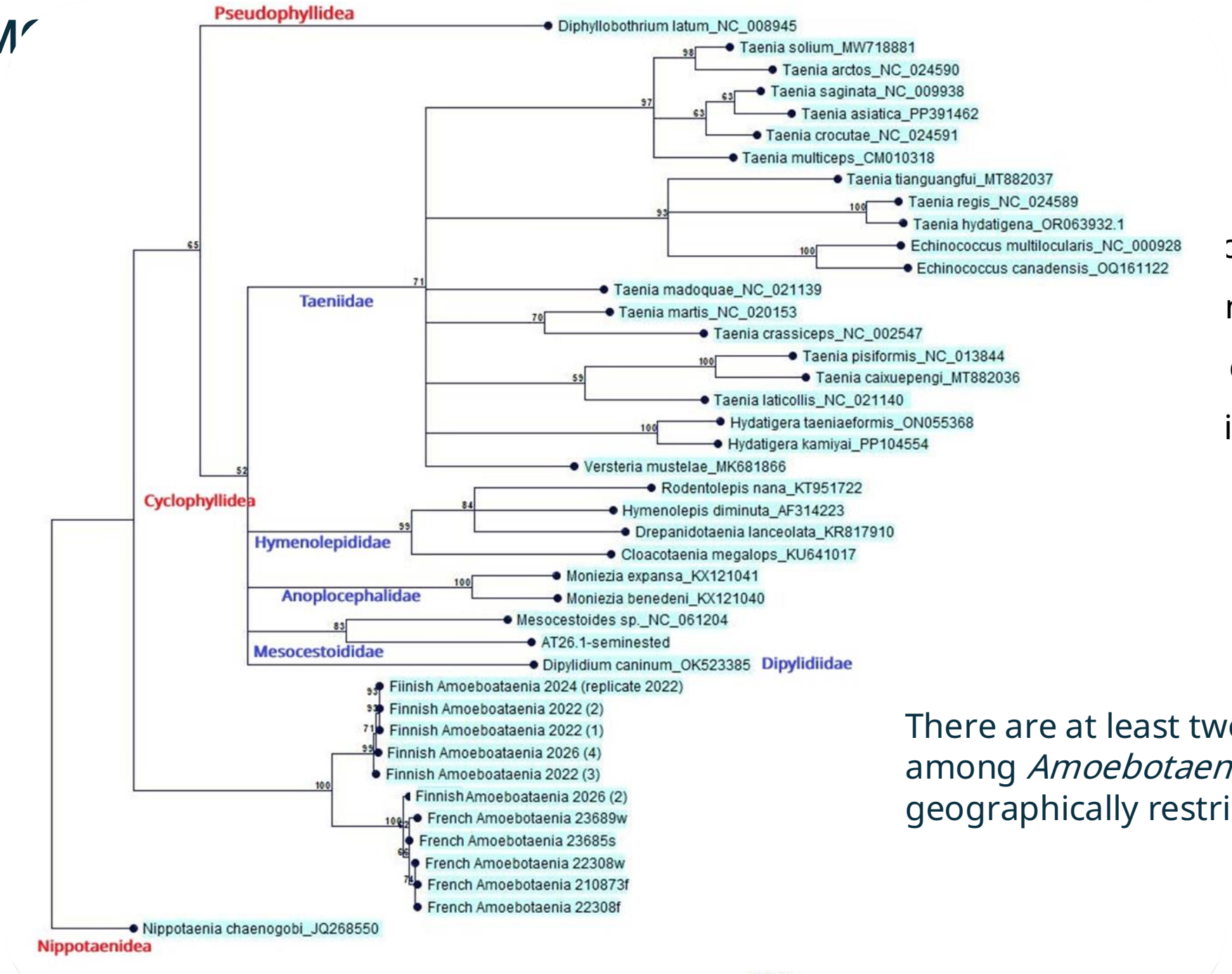


Schéma original d'*E. multilocularis* d'après Leuckart (1853) et schéma original d'*Amoebotaenia paradoxa* d'après Petavy et Deblock (1980).

AM



cimens.

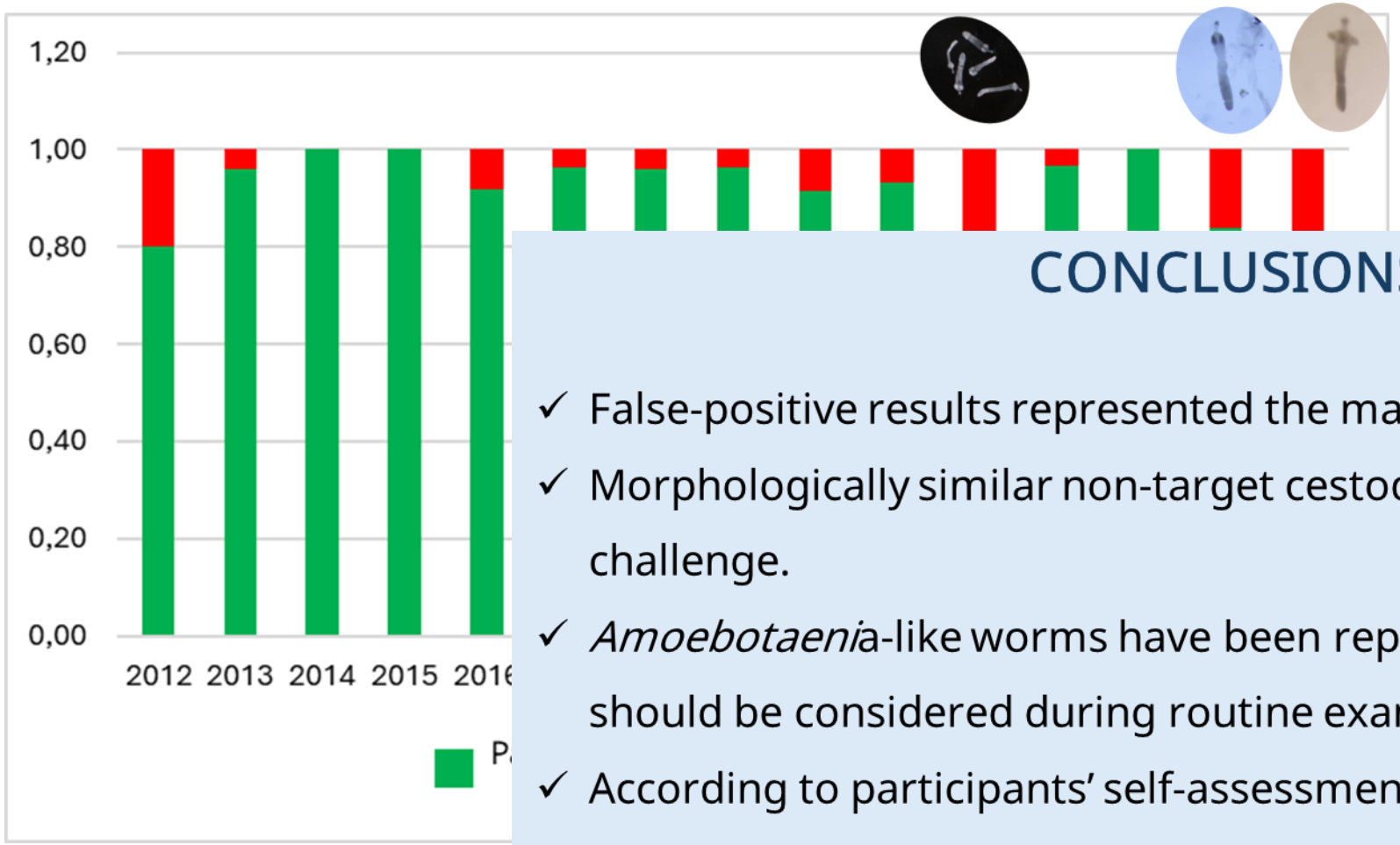
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cosa, whereas

ied with *cox1* also)

There are at least two distinct lineages among *Amoebotaenia*-like cestodes, not geographically restricted.

TREND OVER YEARS



CONCLUSIONS

- ✓ False-positive results represented the main source of error in PT-05 2026.
- ✓ Morphologically similar non-target cestodes remain a major diagnostic challenge.
- ✓ *Amoebotaeni*a-like worms have been repeatedly detected in PT panels and should be considered during routine examinations.
- ✓ According to participants' self-assessment, limited experience was the most frequently reported factor contributing to incorrect results.
- ✓ Morphological expertise remains critical for accurate identification.

THE FUTURE OF PT-05

The availability of material, particularly *Echinococcus* worms, remains a critical issue.



The availability of sufficient batches of good worms cannot always be guaranteed.

- **Call for contributions**: network members able to provide reference material are encouraged to support future PT rounds.
- **Alternative PT frequency**: should the PT be organised on a biennial (2-year) basis rather than annually? We welcome your feedback.

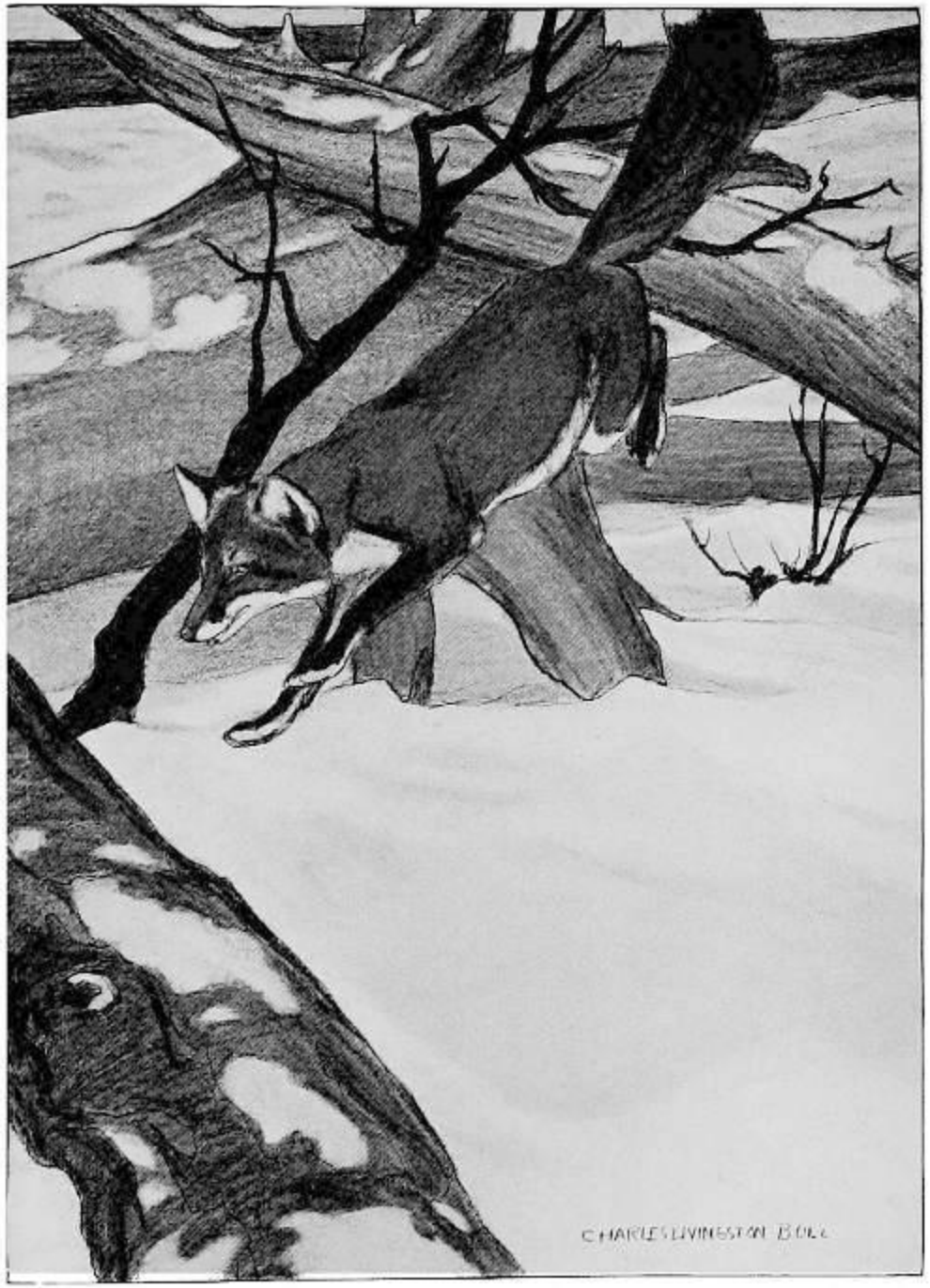


Illustration by Charles Livingston Bull

Thanks for your attention

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