Detection of *Echinococcus spp*. and other taeniid species in lettuces and berries: two international multicentre studies from the MEmE project.

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MEmE:

Multi-centre study on *E. multilocularis* and *E. granulosus s.l.* in Europe: development and harmonisation of diagnostic methods in the food chain

International multicentre collaborative project

20 European countries & 3 international extern partners

Context:

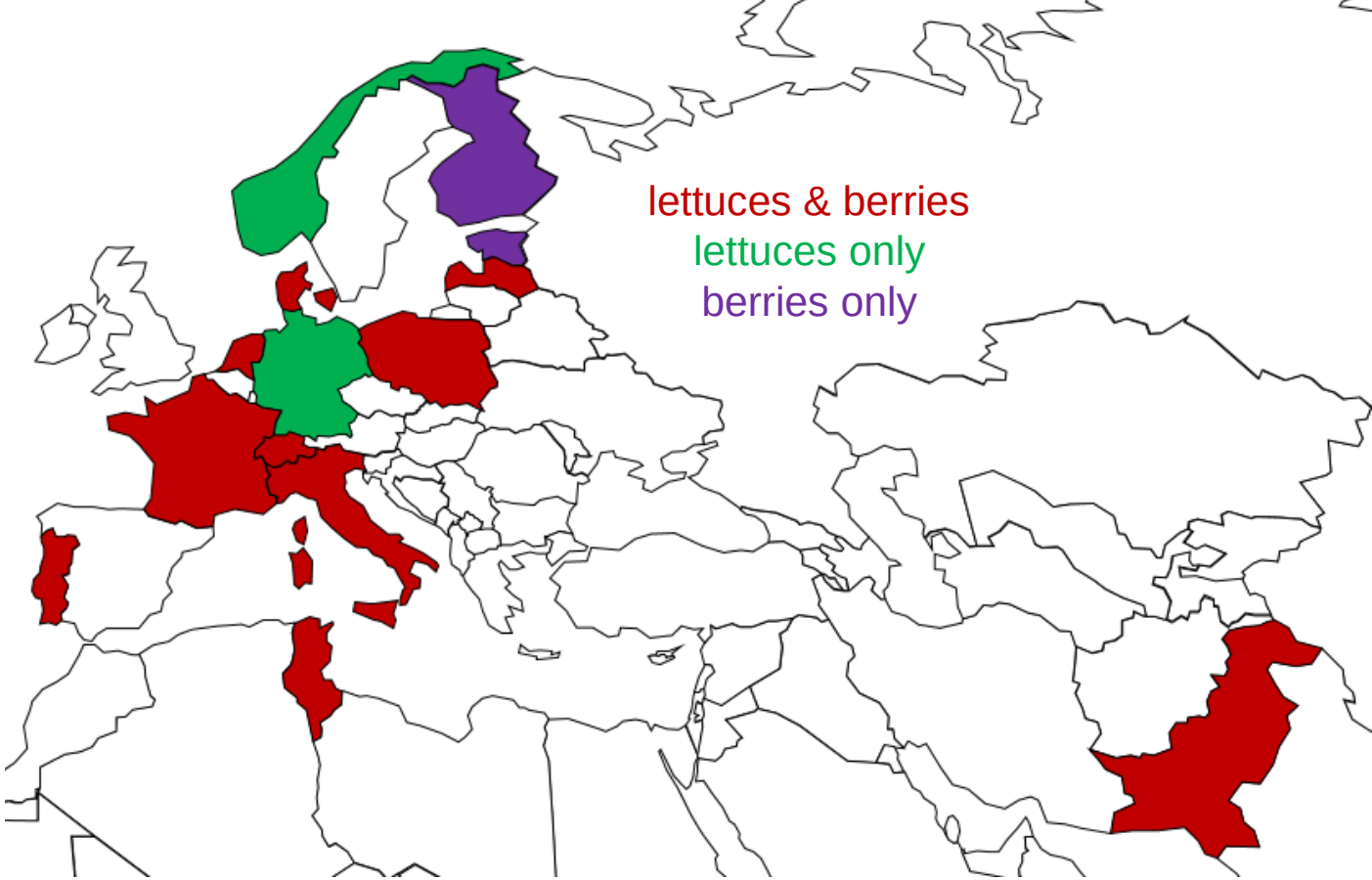
- **Routes of human AE and CE infection** difficult to identify
  - Long asymptomatic period
- **Scarce data** about foodborne transmission
  - Which relative importance ?

Aim:

Provide data about the proportion of lettuces and berries with DNA of Em, Eg and others taeniid species

Sampling

15 European countries
(18 labs)
+ **Tunisia**
+ **Pakistan**



Summer 2021: 1,117 lettuces + 71 other vegetables
(50-100/lab)

Summer 2022: 300 batches of strawberries + 130 blueberries + 50 others
(20-30/lab)

Collected mainly from local markets (wild)
but also private kitchen garden, supermarkets

Methods

Washing

500ml Tween + hand shaking → sedimentation

Sequential sieving

105µm → 40µm → 20µm

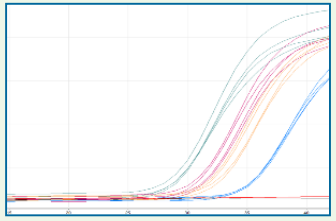
(Guggisberg et al. 2020)

Molecular detection

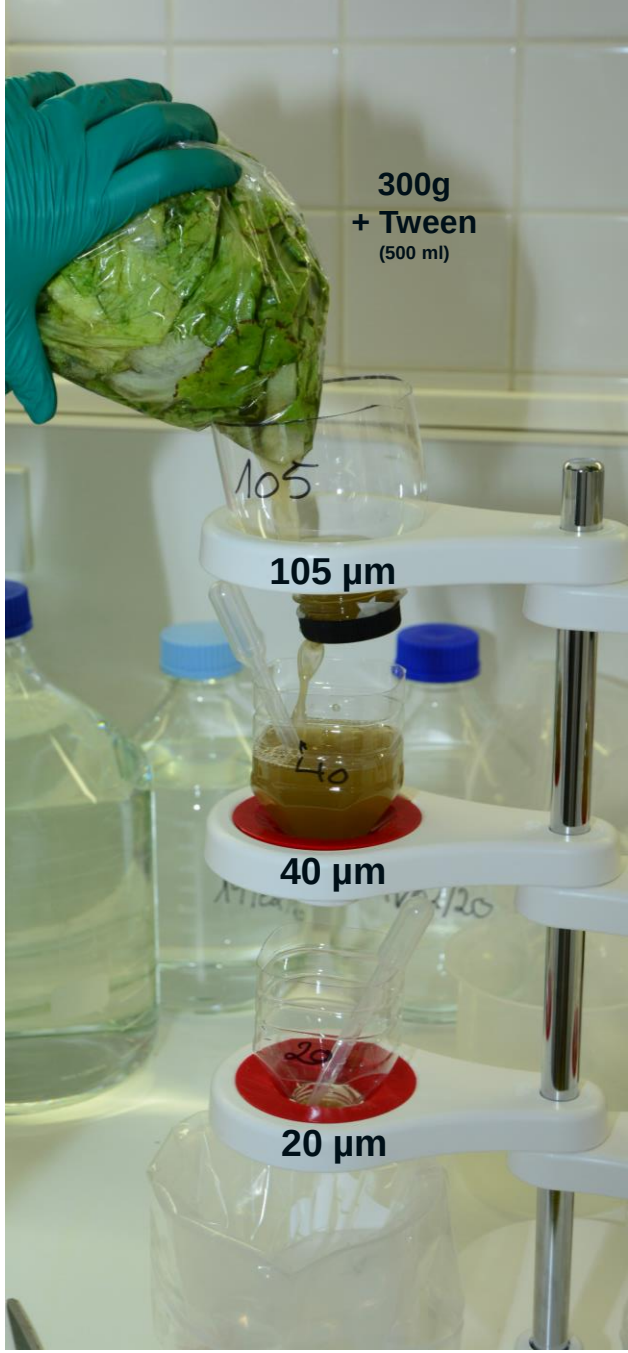
DNA extraction from pellet

Real-time PCR: **Em** (Knapp et al. 2016)

Eg sl (Maksimov et al. 2020)



PCR/sequencing: **other taeniids** (Trachsel et al. 2007)



Limit of detection

Evaluation in Anses lab conditions

n=24

95%

75%

50%

- 2 eggs (5/5) in original method from Guggisberg et al. 2020

n=8

95%

88%

88%

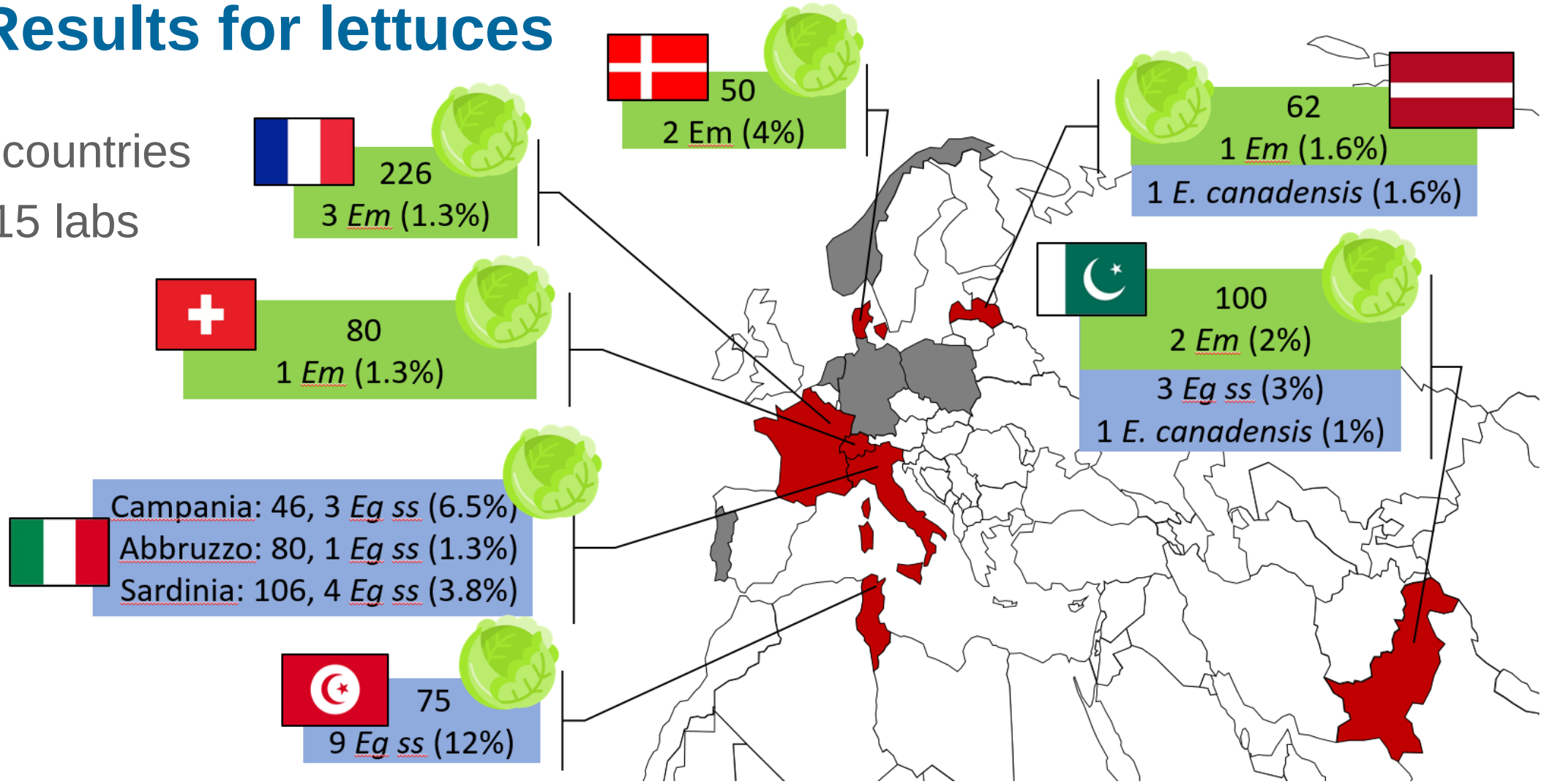
- Not evaluated previously on berries

➔ **Relevant limit of detection**

Results for lettuces

12 countries

15 labs



Global results in European endemic areas:

Em: 1.2% (0,5-2,5) **Eg sl: 1.3%** (0,6-2,4)

Other taeniid: **1.7%** (1-2,7)

Results for berries

12 countries, 12 labs

European endemic areas

Em 5.4% (2.7-9.5)

7.3% (2.7-15.2)

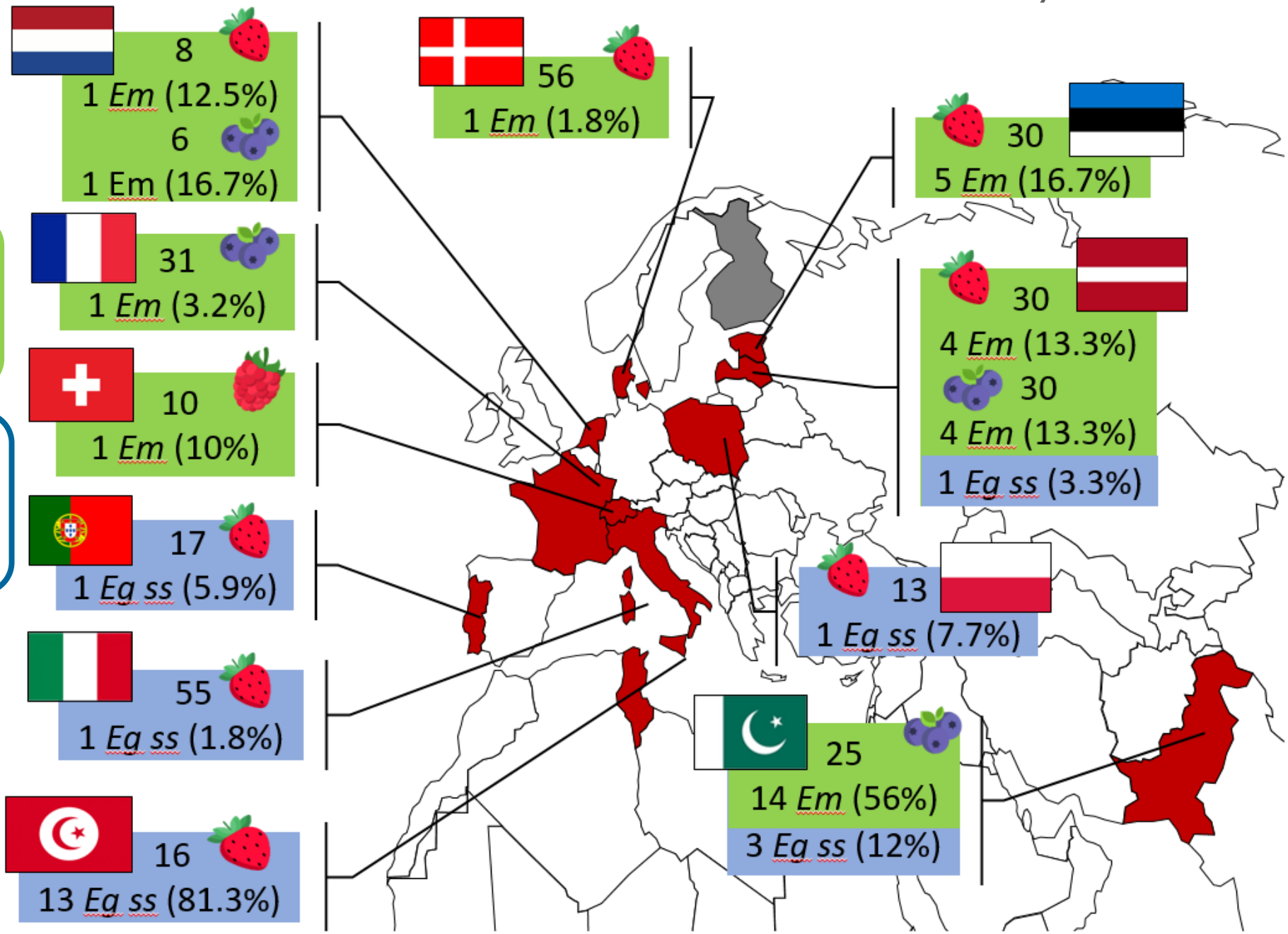
Eg ss 1.5% (0.3-4.3)

1.3% (0.0-6.9)

Other taeniids (all areas)

1.3% (0.4-3.3)

1.5% (0.2-5.4)



Complementary results

- **Vegetables others than lettuces** (n=69): chard, parsley, basil, sorrel, endive, spinach, beet and carrot leaves …
  - ✓**1 chard** from North Germany positive for *E. multilocularis*

- **Others berries** (n=50): raspberries, blackberries, currant, …
  - ✓**1 raspberry** from Switzerland positive for *E. multilocularis*

- **Other taeniid species detected**
  - ✓mainly *Hydatigera sp*. and *T. hydatigena*

✓1.7%

✓2.5%

- **Global proportion of DNA from all Taenidae species** (Em, Eg, taeniid)
  - ✓**5.4%** in lettuces
  - ✓**11.9%** in berries

Transfer of taeniid eggs from feces to food is not a rare event

Discussion

- **One step more in the long road to evaluate the role of human foodborne contamination by *Echinococcus* spp.**

- **Realization of only washing step by each participants**
  - ✓ **Illustrated SOP** (lettuces and berries)
  - ✓ increases **nb of participants** (no new method to perform)
  - ✓ Facilitate process of fresh samples

- **Filtration and molecular detection in one lab**
  - ✓ **Easily comparable results**: same method
  - ✓ **Great confidence and robustness** in the results: LOD, reproducibility

Discussion

High proportions of *Em* and *Eg* in lettuces and berries in Europe

- ✓ Even higher in Tunisia and Pakistan
- ✓ In accordance with known high endemic areas
- ✓ Quite similar proportions between European countries
- ✓ Berries significantly more contaminated than lettuces for *E. multilocularis*

Detection of DNA but:

➢ No observation of eggs
- ✓ But low sensitivity VS molecular biology
- ✓ DNA supposed to be from eggs regarding the method

➢ Estimation of the number of *Echinococcus* eggs (dPCR)
- ✓ According LOD: **generally 1 to 3 eggs in positive food samples**

No proof of viability of the eggs

Acknowledgments

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