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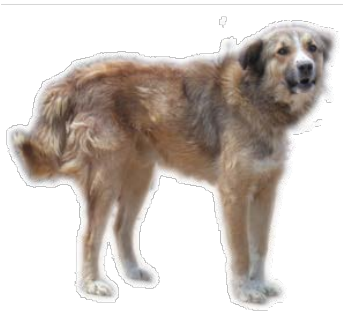
ANSES Nancy National Reference Laboratory for Echinococcus spp.

WHO collaborating centre for research on zoonosis

WOHA reference laboratory for rabies

European Union reference Laboratory for rabies

NRL for rabies and echinococcosis for agriculture ministry



From field to fork: contamination of lettuces and berries by *Echinococcus multilocularis*, *Echinococcus granulosus sensu lato* and other Taenidae species eggs in Europe and beyond

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ne HEALTH EJP MEME

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Introduction

Alveolar and Cystic Echinococcosis:

- Due to **accidental ingestion of eggs (30µm)** in the environment
- **Routes of human infection** difficult to identify
  - Food, hand-to-mouth, …
  - long asymptomatic period (up to 15 years)

Foodborne transmission: most important

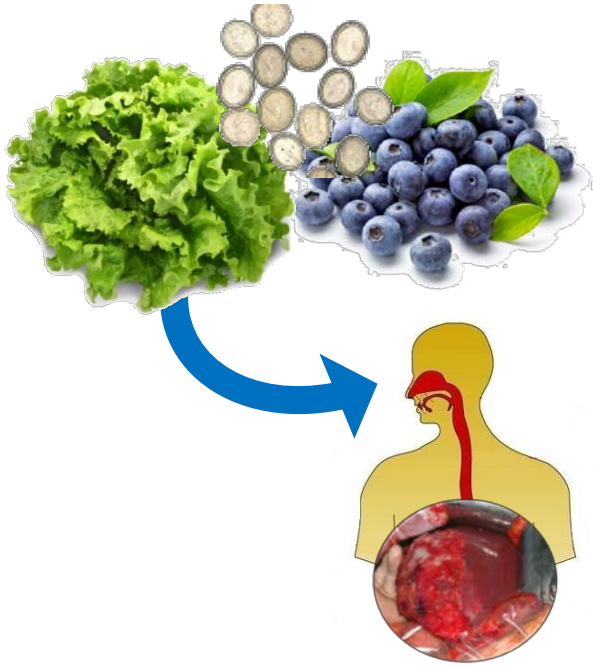
- But **scarce data available** on hand-to-mouth route
- Great need of data from food: especially from **fruits and vegetables**
- Cultivated areas (private, professional):

  hotspots for both fox and cat defecation

  (Bastien et al. 2018)

Aims of the study :

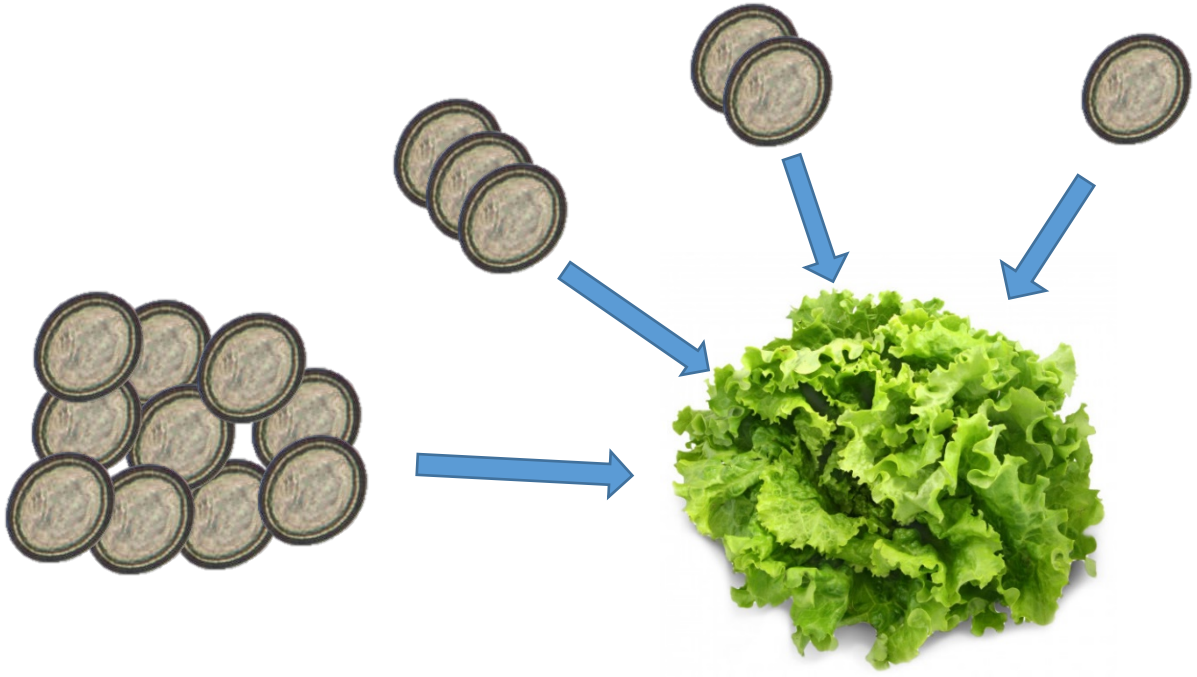
- **Produce data to evaluate proportion of lettuces and berries with *Echinococcus* and others Taenidae species eggs**



- **Methods**
  - ➢ Transfer of a newly developed method (Guggisberg et al. 2020)
  - ➢ Evaluation of the limit of detection
  - ➢ Sampling Lettuces in Europe in the context of EJP MEME

Materials and methods

- **Estimation of the limit of detection**
  - Lettuces from supermarket
  - Spiked with known number of Em eggs
    - ➢ Produced by experimental infection of fox (EJP MEME)



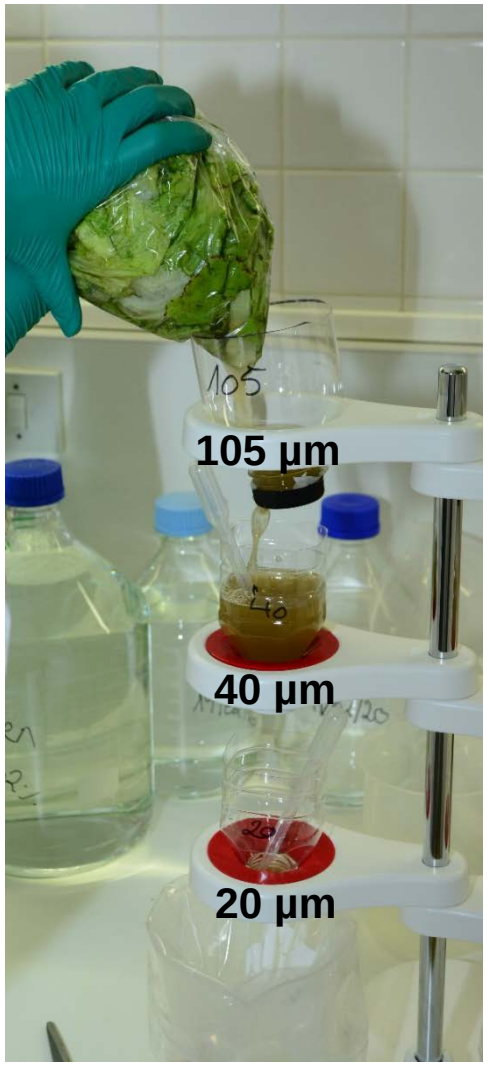
Materials and methods

- **Concentration of eggs**
  - Sequential sieving

    from Guggisberg et al. 2020

300g max
by lettuce

Washing
500ml of Tween

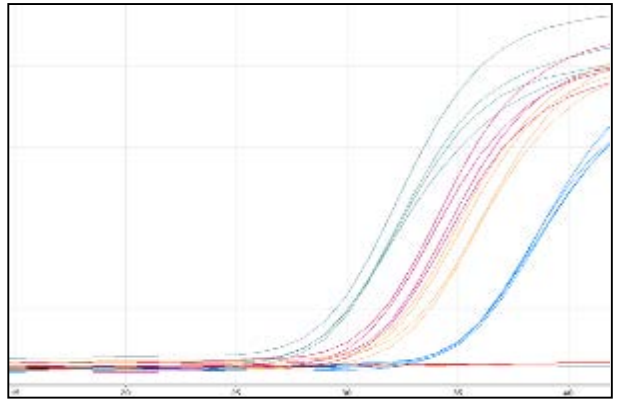


Sequential sieving

Tween washing
filter mesh 20µm

Materials and methods

- **DNA extraction**
  - kit for tissue (Qiagen)
- **Molecular detection**
  - *E. multilocularis*:
    - ➢ specific qPCR (Knapp et al. 2014)



Results

- **Limit of detection**
  - determinded by testing 24 lettuces spikes with known eggs number

95%

75%

50%

WP3-T6. Contamination of vegetables for human consumption by Em/Eg

ST1 contamination of vegetables

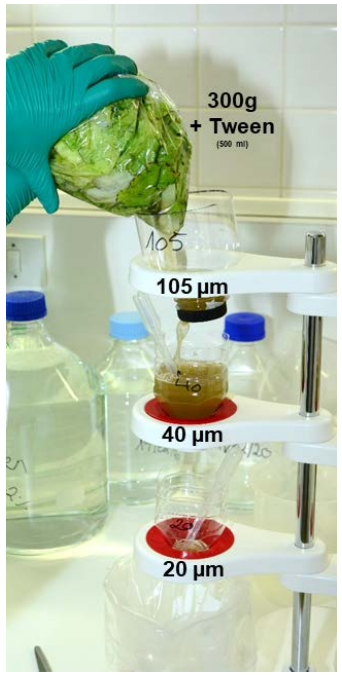
- **Lettuces sampling from local markets and supermarkets**
  - High Em and Eg endemic areas from EJP MEME partners
  - Vegetables were collected during summers 2021-2022
  - 50/100 vegetables samples collected by each partner
  - The first washing step of the method perform by partners
  - Standard Operating Procedure provides instructions for washing step
  - Pellets were frozen and transferred to Nancy for filtration and molecular analyses

- **15 labs from 12 countries**
- **1,034 lettuces (~50-100 by country)**

Methods perform in Nancy

Sequential sieving

- Pellets received from partners

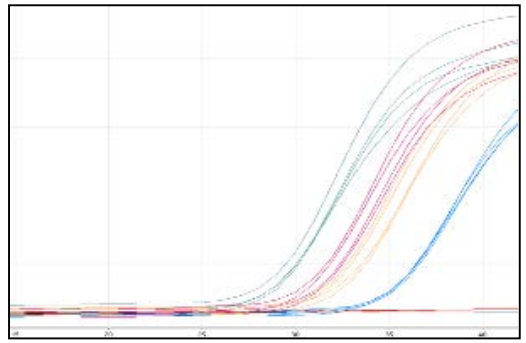


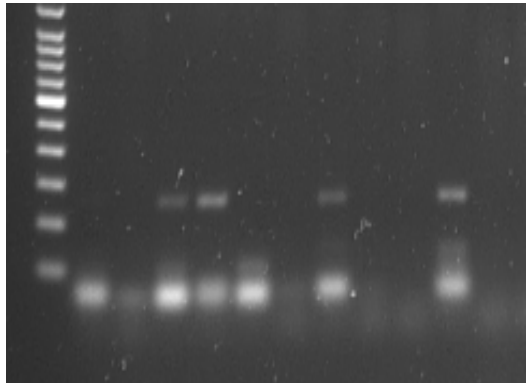
DNA extraction

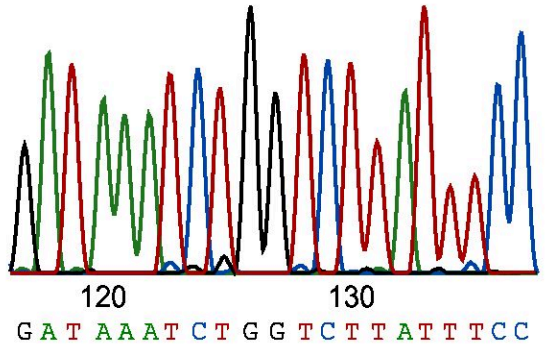
- kit for tissue (Qiagen)

Molecular detection of taeniid eggs

- *Echinococcus* specific real-time PCR
  - ✓ ***E. multilocularis*** (Knapp et al. 2014)
  - ✓ ***E. granulsous sl*** multiplex (Maksimov et al. 2021)
- End point PCR for **others taenias** (Trachsel et al. 2007)







Results

E. multilocularis: from 714 lettuces (from endemic areas)

✓ 1%: n=7 (1 case: FR, SW, LV; 2 cases: DK, PK)

E. granulosus sl: all countries are endemic

✓ 2.2%: n=23 *E. granulosus ss*: IT (3.5%), PK (3%), *E. canadensis*: 1 in LV, PK, CH

Others Taenidae species:

✓ 2.5%: n=26 mainly ***Hydatigera sp***. in Europe (FR, CH, DE, IT, NO, LV+PK)

T. hydatigena in *E. granulosus ss* high endemic areas (IT, PK)

but also *T. saginata*, *T. multiceps*, *T. krabbei/serialis*, …

WP3-T6. Contamination of vegetables for human consumption by Em/Eg

ST2 contamination of berries

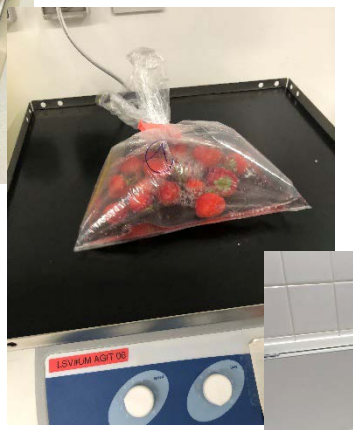
- **Detection of Echinococcus spp. eggs in strawberries and blueberries sampled from local markets and supermarkets**
  - Filtration method validation on strawberries with **95% probability** of detection of **three eggs** in 200g sample

  - 100/200g berries samples collected by each partner
  - From 20 to 30 samples per country
  - The first washing step of the method perform by partners
  - Standard Operating Procedure provides instructions
  - Pellets were frozen and transferred to Nancy for filtration and molecular analyses

- **11 labs from 11 countries**
- **42 berries samples (ongoing ~20 to 30 by country)**

Results (preliminary)

- ***E. multilocularis*: from France**
  - ✓ Strawberries: 0 %, n=0 (11)
  - ✓ Blueberries: 3.2%, n=1 (31)



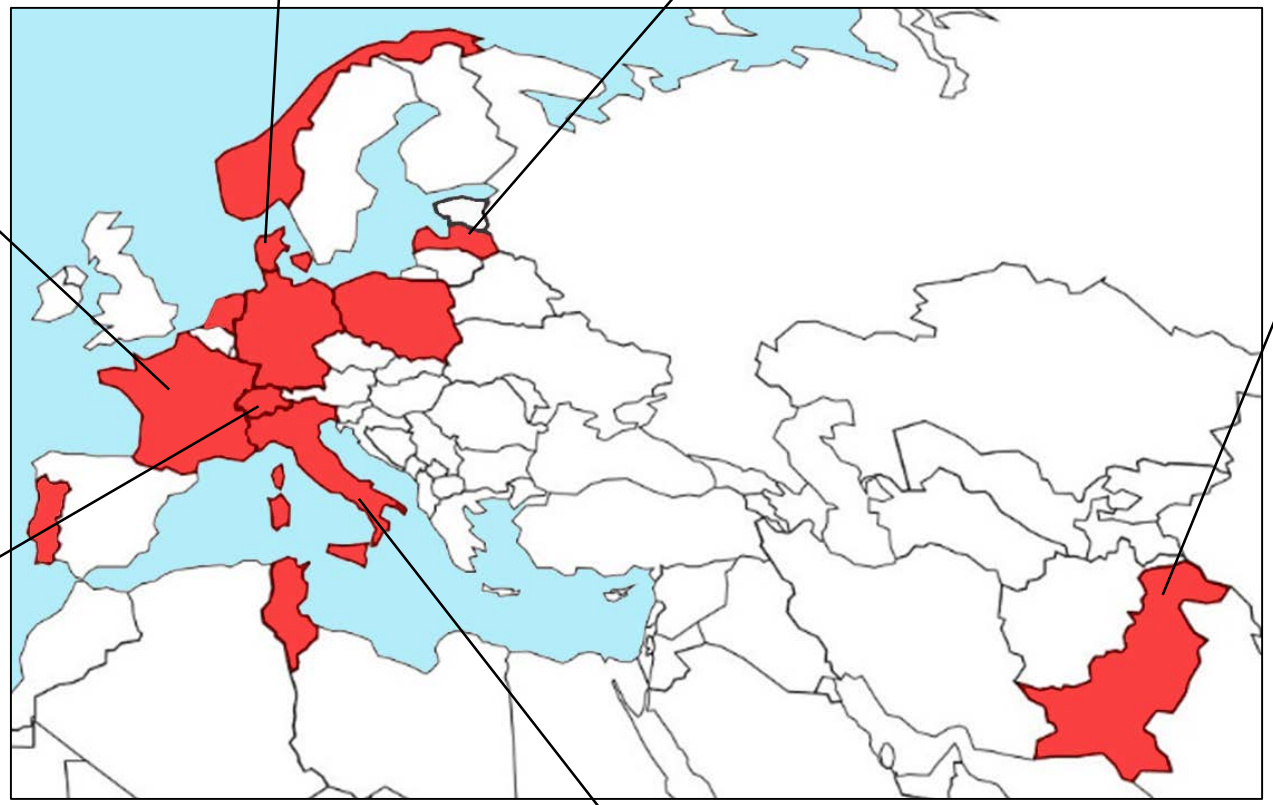
- ***E. granulosus sl*:**

Ongoing

- **Others Taenidae species:**

Ongoing

Conclusion



50 lettuces
2 Em

62 lettuces
1 Em
1 E canadensis

144 lettuces
1 Em
42 Berries
1 Em

100 lettuces
2 Em
3 Eg ss
1 E canadensis

80 lettuces
1 Em
1 E canadensis

Roma: 80 lettuces, 1 Eg ss
Sardenia : 105 lettuces, 4 Eg ss
Napoli: 46 lettuces, 3 Eg ss

Discussion

High proportion of lettuces with taenid eggs (5.7%)

- ✓ Including **zoonotic *Echinococcus* species**
- ✓ Coherent with known high endemic areas
- ✓ **Potential source of human infection**
  - But egg's viability remain uncertain
  - More data required (others vegetables: parsley, spinach, chard …)

Proportion variable of berries with taenid eggs

- ✓ France 1 Em / 42 samples
- ✓ Including **zoonotic *Echinococcus* species**

Ongoing

- ✓ **Berries**: 12 countries, sampling to be finished
- ✓ Lettuces & berries: detection of ***T. gondi***, ***Crypto***, ***Giardia***

This largest epidemiological study ever conducted on contamination of vegetables by Em and Eg which will contribute to the understanding of foodborne transmission.

Thank you for your attention