Food contamination by *Echinococcus* and other taeniid species is essentially due to only several eggs according to digital PCR estimation

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Echinococcus as foodborne parasites

E. granulosus and *E. multilocularis* among the four most important

Availability of data from food : only since 2015

- 13 studies

For years, problem of method sensibility:

- **Microscopy**: observation of eggs
- **Molecular biology**: DNA supposed from eggs

Presence of Echinococcus in food

Mainly data from Europe

- *E. multilocularis*

0-4%

0-7%

E. multilocularis in **lettuces** from European endemic areas: **1%**
(Guggisberg et al. 2020, Barlaam et al. 2021, Umhang et al. 2025)

- Detection in high and low endemic areas

E. multilocularis in **other types of vegetables** (mainly chards, parsley and spinach) : **1.8%**

Generally, higher proportion of Em/Eg in berries VS lettuces (Umhang et al. 2025)

Questions & Aims

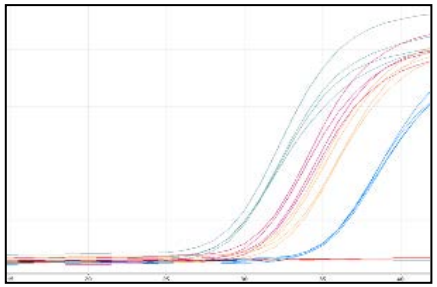
What is the number of eggs concerned by contamination of one food item?

- Evaluate **number of copies of mtDNA** in one taeniid egg
- Obtain first **molecular evaluation of number of eggs** using digital PCR

Is microscopy really far less sensitive compared to molecular tools?

- Realize a **performance comparison** of methods for both qualitative and quantitative detection of taeniid eggs in food matrix.

Methods



Real-time PCR (qPCR):

E. multilocularis, *E. granulosus ss*, *Taenia sp.*

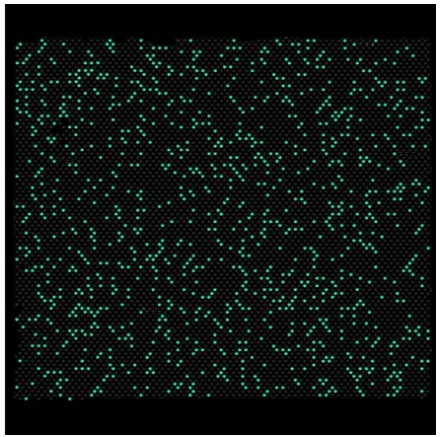
(Isaksson et al. 2014, Maksimov et al. 2020, Oberli et al. 2023)

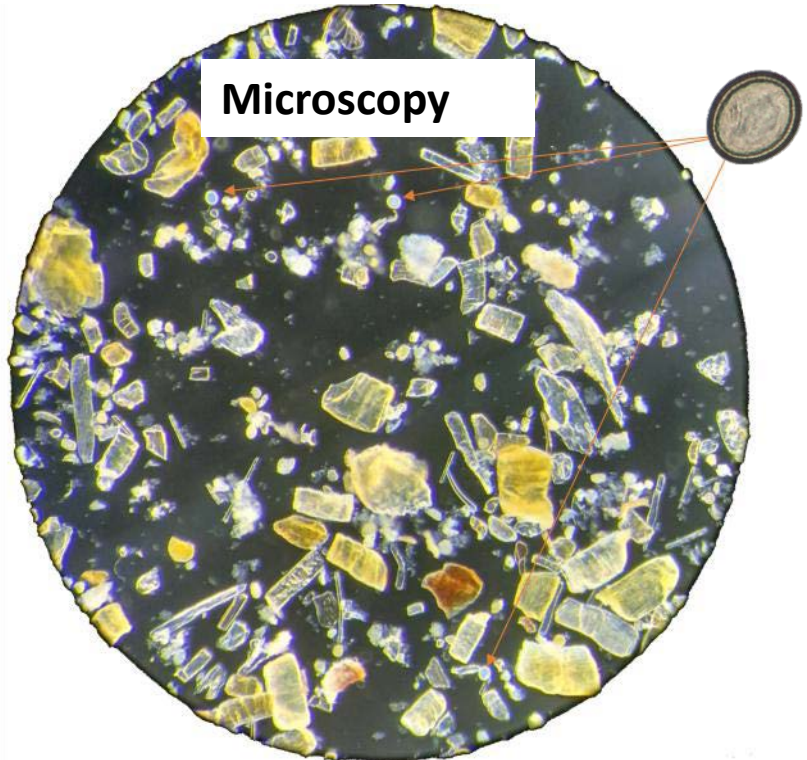
Digital PCR (dPCR): as qPCR (QUIACUITY)

No calibration curve,

absolute quantification,

accurate estimation





Number of mtDNA copies in one egg

Isolation of eggs from **feces of sheep dogs collected in the field in Greece**:
(-80°C, + several freezing and thawing)

E. granulosus s.s.

- 1 stool → 10 eggs
- **3445** copies (+/-1179)

T. hydatigena

- 3 stools → 19 eggs (9/5/5)
- **3017** (+/-1495) to **3418** copies (+/-2399)

Very low number regarding previous data of 7100 (+/-1900) (Trachsel et al. 2007)

➢ Most probably due to **storage conditions**

Number of mtDNA copies in one egg

Isolation of ***E. multilocularis*** eggs from **fox experimentally infected**:

First infection:

- one stool (-80°C), 10 eggs
  - ✓ **5182** copies (+/- 1556)

Second infection:

- 1st stool (-80°C), 7 eggs
  - ✓ **5244** copies (+/-1438)
- 2nd stool, 10 **viable eggs**
  - ✓ **4940** copies (+/-1005)

Average value of mtDNA copies in one *E. multilocularis* egg

5109 (+/-1362)

Inferior to previous value: better estimation of dPCR vs qPCR

Value used as reference for all type of taeniid eggs

Contamination of food samples

47 positive DNA samples from **MEmE project** (Umhang et al. 2025)

- Lettuces: 12
- Berries: 34
- Chard: 1

- *E. multilocularis*: 23
- *E. granulosus s.s.*: 21
- Other taenids: 3

Food contamination by

1 to 5 eggs: 95.7%

only 1 egg: 83%

Only 2 samples >5 eggs: 7 (blueberries, Pakistan) and 10 eggs (chard, Germany)

Large majority of food contamination due to only several taeniid eggs

Microscopy VS Molecular Biology

Food pellets

- washing/filtration of lettuces from supermarkets
- pellets pooled and divided
  - **15 aliquots** of 200 µl
- **spiked with 0 to 63 *E. multilocularis* eggs**

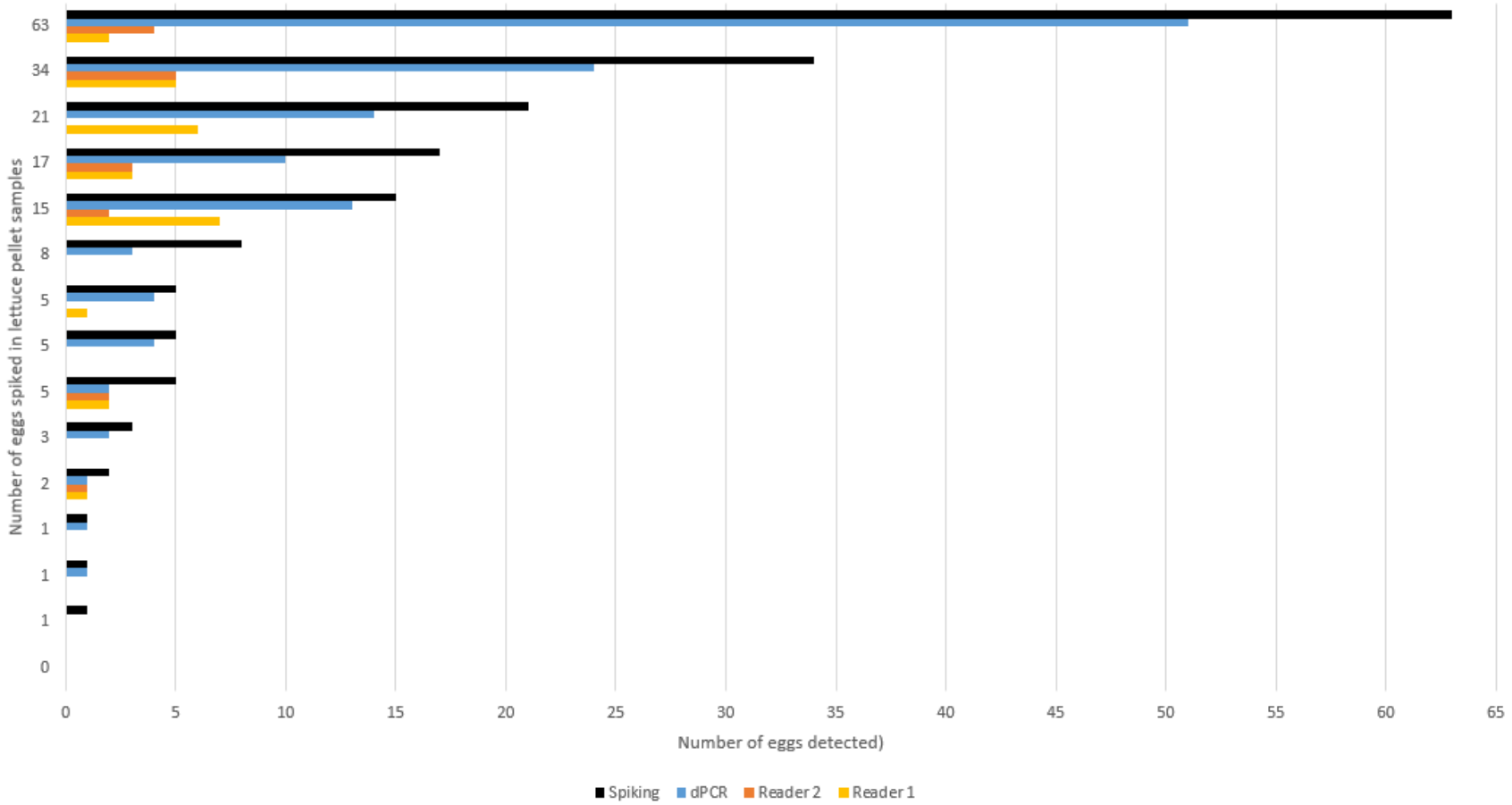
Microscopy

- **blind** observation
- around 1 hour/sample
- 2 independent skilled observers

Molecular biology

- transfer pellet for DNA extraction
- **qPCR** for qualitative detection (+/-)
- **dPCR** for quantitative estimation (nb eggs)

Microscopy VS Molecular Biology



No egg detected in the negative sample in all methods

Microscopy VS Molecular Biology

Sensibility	All samples 1 to 63 eggs	1-5 eggs	Described contamination (95% ≤ 5 eggs)
Microscopy	50%	31%	40%
qPCR	**93%**	**88%**	**88%**

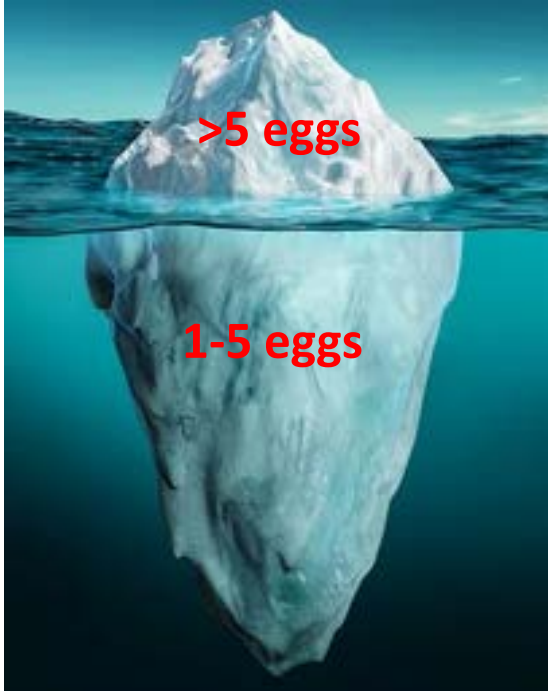
Superior sensibility by qPCR detection

More accurate estimation of egg number by dPCR

Quantitative estimation	MAE Mean Absolute Error	MARE Mean Absolute Relative Error
Microscopy	10 eggs	84%
dPCR	4 eggs	36%

Better performance of molecular methods for both detection and quantification

Discussion



Only two studies with taeniid eggs estimation:

- Turkey: 7 samples, **4 to 68 eggs** (median 12) (Kozan et al. 2005)
- Tunisia: 3 samples, **41, 56, 72** (M'Rad et al. 2020)

➢ Only high number of eggs observed:
due to use of low sensistivity of microscopy

➢ Explain higher proportion of food contamination reported with PCR/qPCR

Is 1 to 5 eggs enough to develop infection in humans?

Not systematic with 100 eggs in mice (Federer et al. 2015)

Real need to estimate **viability** of *Echinococcus* eggs in food to go further in foodborne risk evaluation

Thank you for your attention

and to all my collaborators

Questions ?