



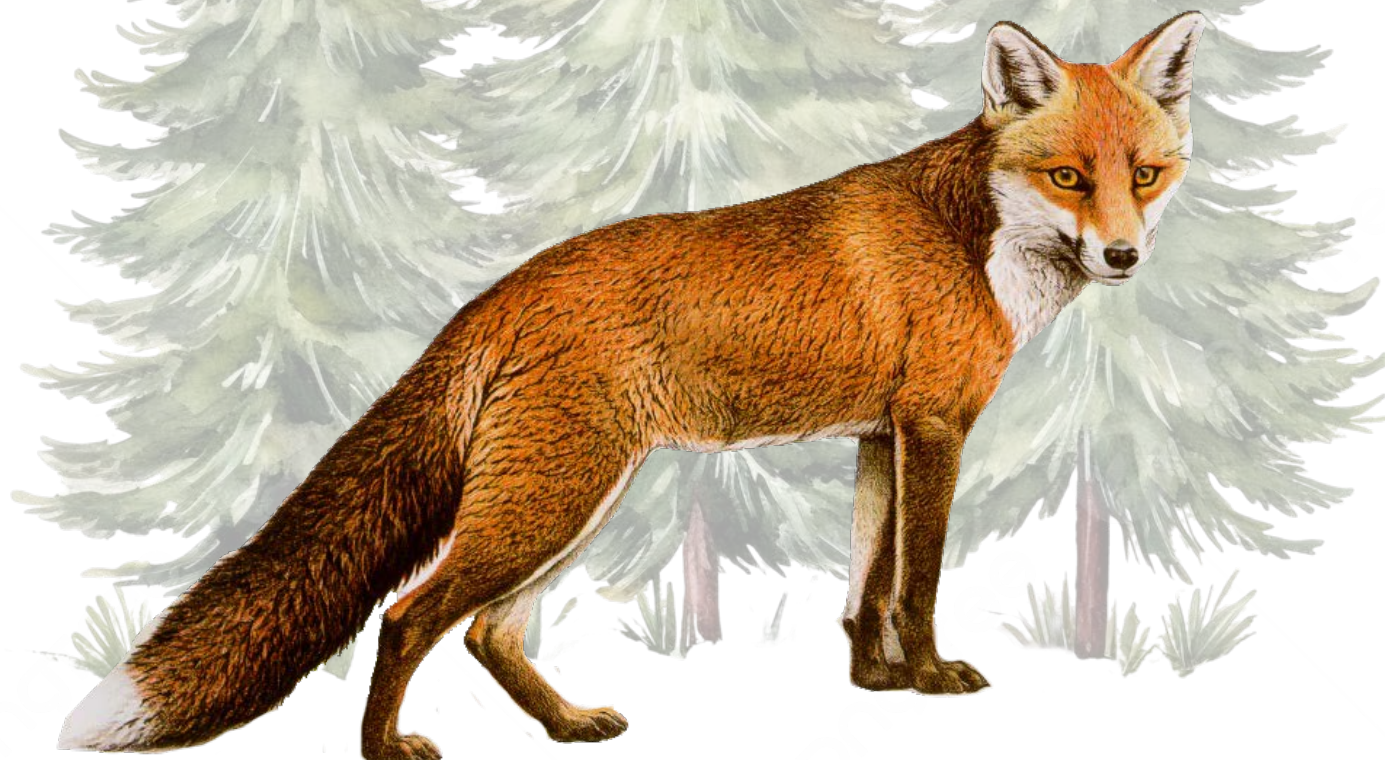
National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

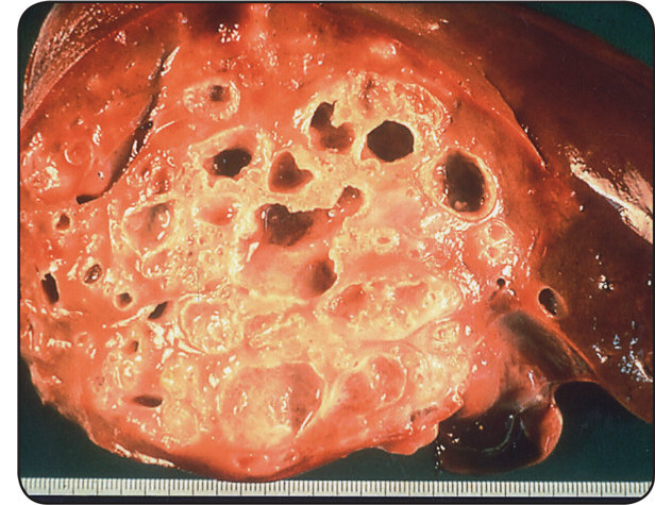
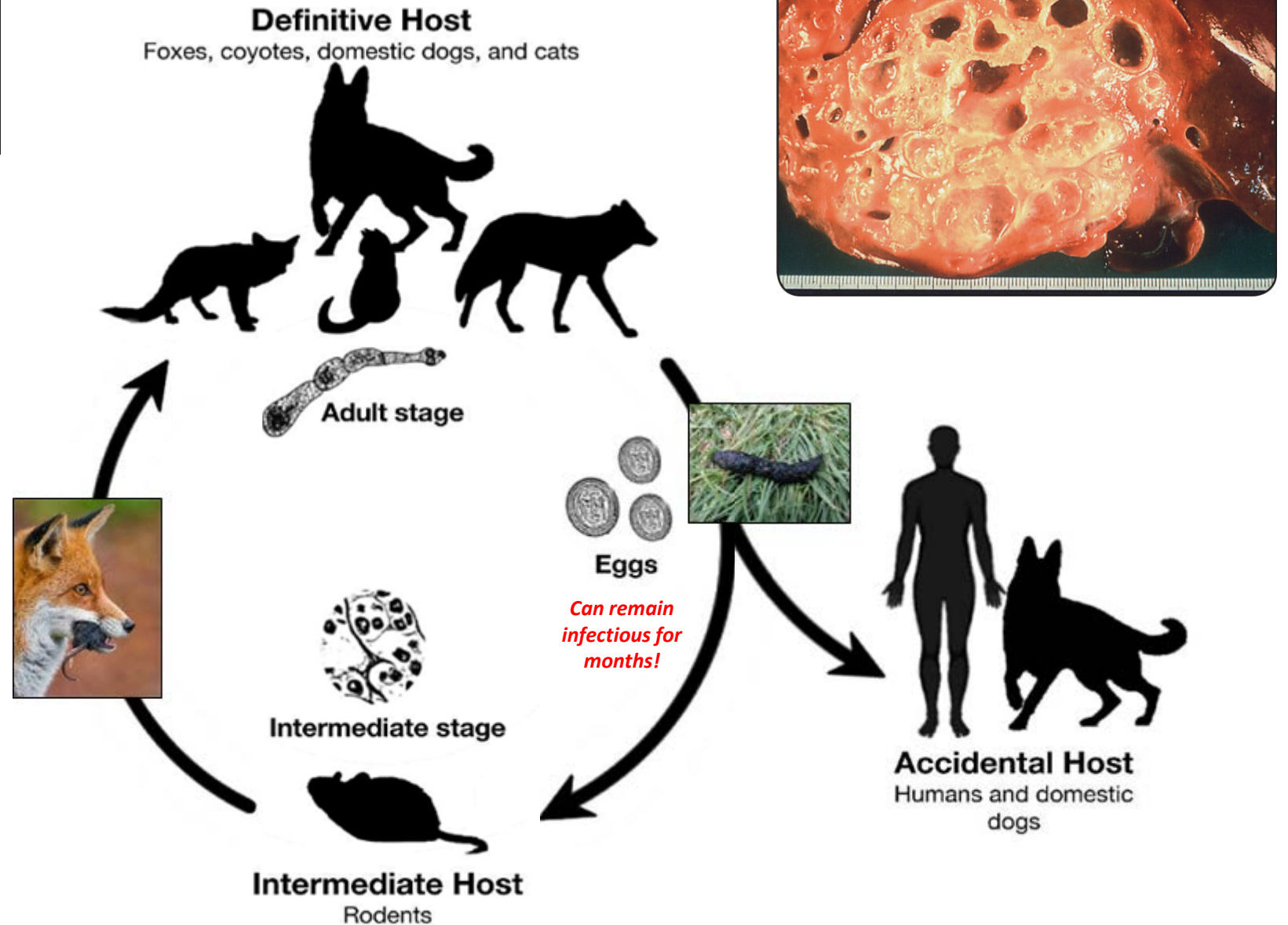
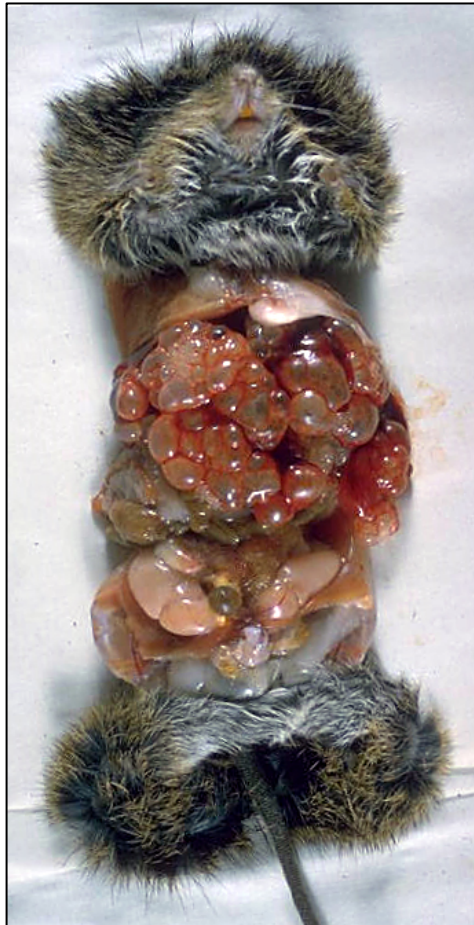


***E. multilocularis* contamination of commercially grown berries and infection of foxes in the Dutch province of Limburg**

**Marieke Opsteegh
Laura Derks**

EURL-P workshop 2025

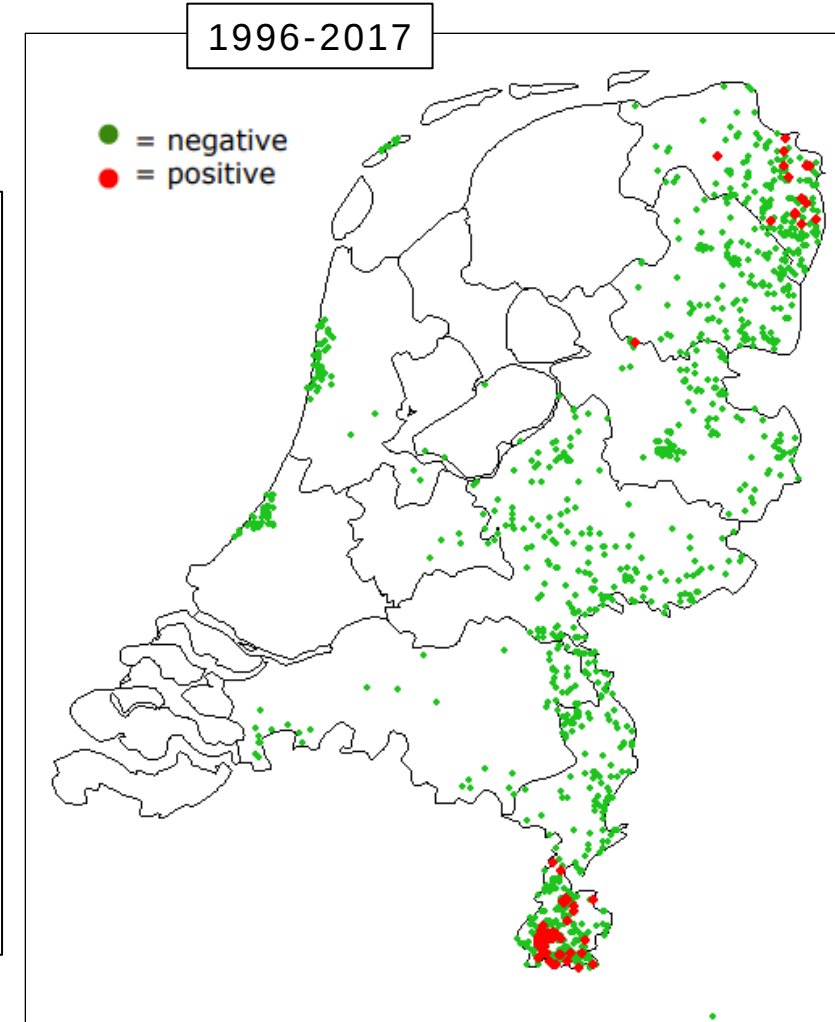
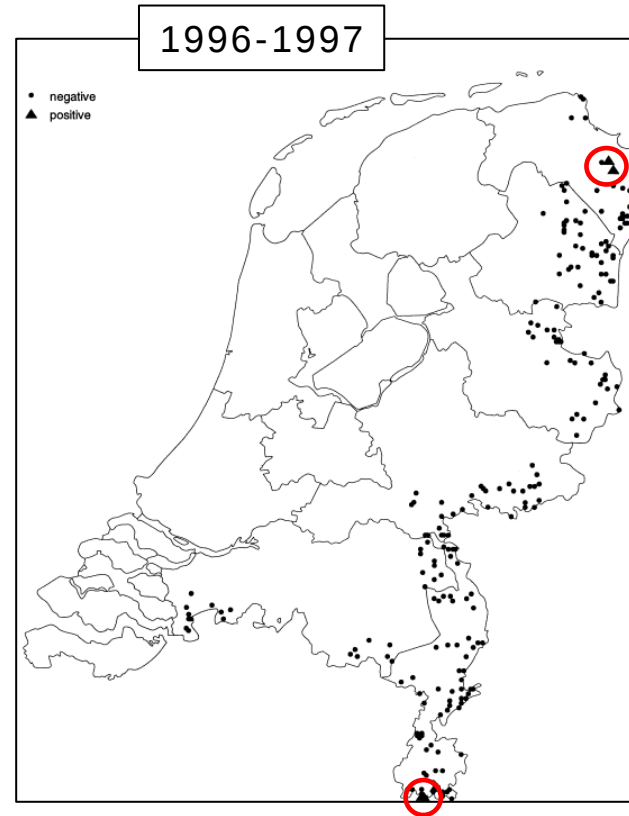






Situation in The Netherlands

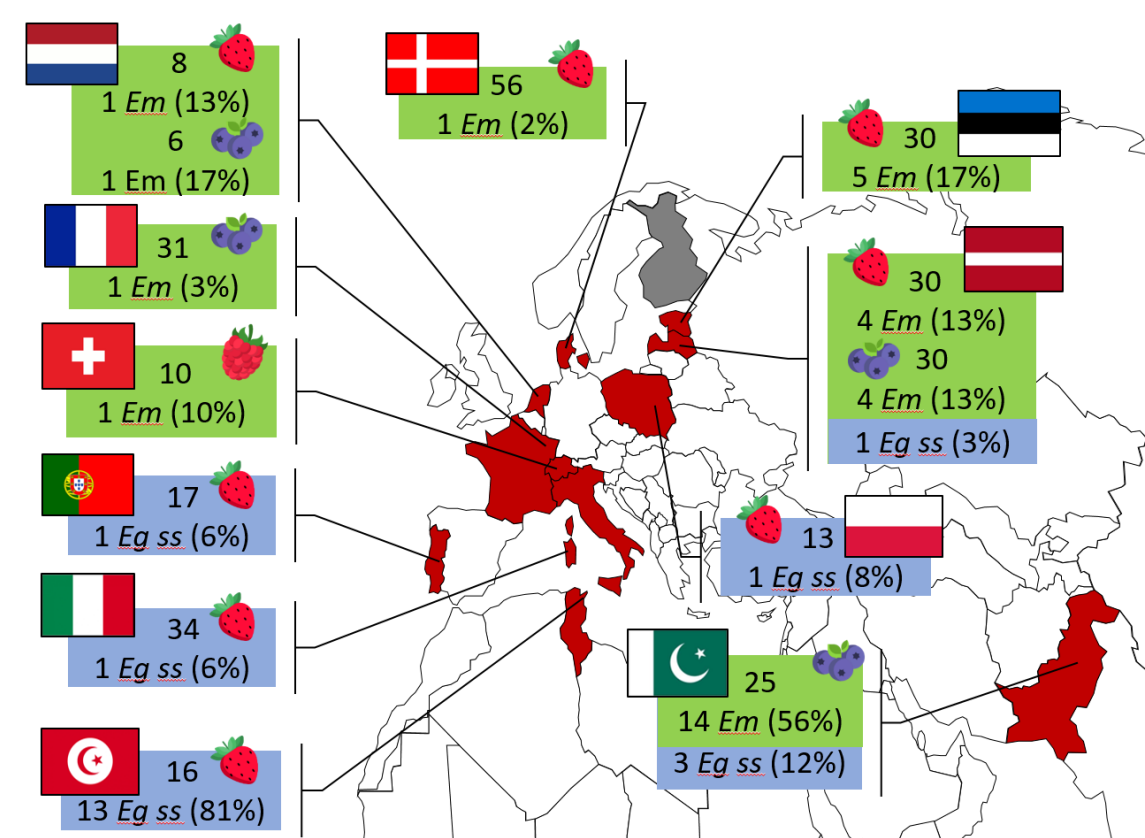
- › 1996: first fox study
- › Growing endemic area
- › Strong increase in Em prevalence in foxes
 - Maastricht area: 11% to 59% (2006-2014)
- › First human case in 2008





Why now?

- › MEmE (2021-2022) results
- › 2/14 positive in Limburg
 - Strawberries and blueberries
 - Roadside food stalls
- › Need for follow-up study
 - More samples
 - Known origin
 - Risk factor analysis



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

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PhD project Laura Derks

1. What is the **contamination rate** of *E. multilocularis* on **outdoor commercially** grown berries in the endemic area of Limburg?
2. What is the current **prevalence** and **endemic area** of *E. multilocularis* in **wild foxes** in Limburg?
3. Are there significant differences in human **seroprevalence** within and outside of the endemic areas?
4. Is *E. multilocularis* in The Netherlands **genetically** related to *E. multilocularis* in other European countries? (genetic origin)



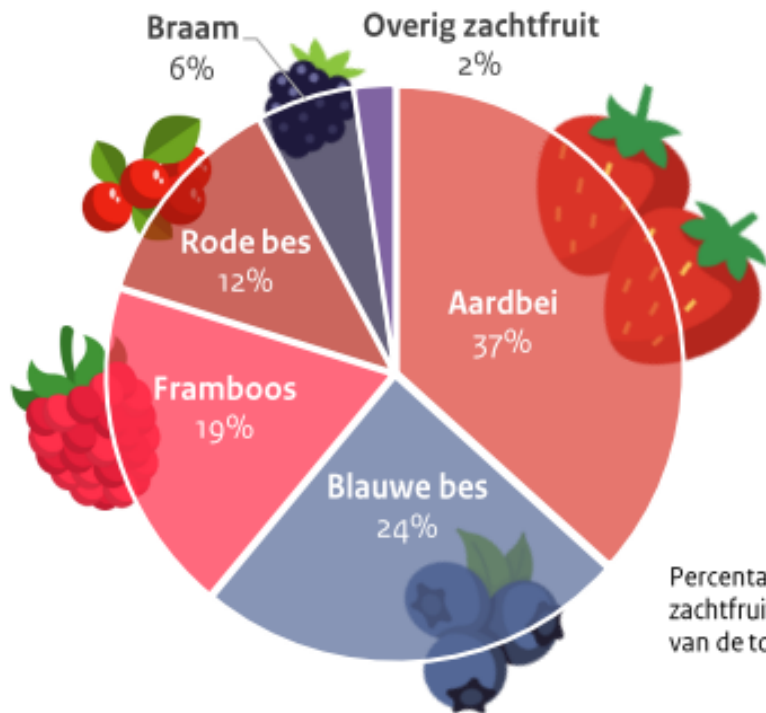
Fruit sampling

- › South and Central Limburg
- › 22 plots on 14 soft fruit farms
- › 2 visits during harvest season
- › 3 sample types
- › Questionnaire
- › Camera traps





Types of farms



Percentage verzamelde zachtfruitmonsters per fruitsoort, van de totaal 220 monsters.





Lab analysis

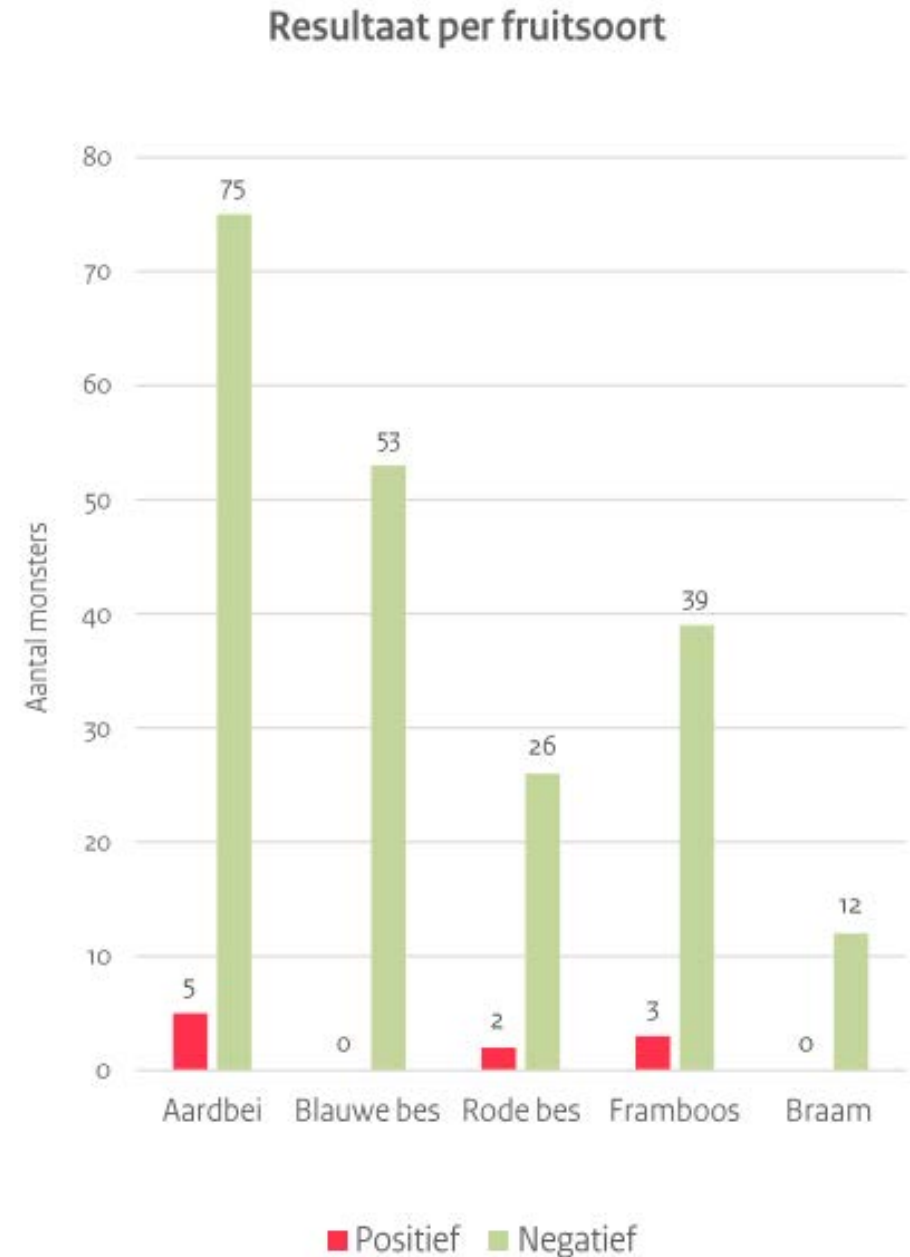
- › Fresh fruit processed within 3 days
- › Washing and centrifugation → freeze pellets
- › DNA isolation (DNEasy Blood & Tissue Kit®) and inhibitor removal kit
- › Specific *E. multilocularis* qPCR (12S mitochondrial gene)





Results fruit study

- › **Fruit samples (220):** 10 qPCR-positive (4.5%)
 - 5 / 14 farms (3 South, 2 Central Limburg)
 - None of the farms tested at both visits
 - Lots of variation in growing methods
 - Very low DNA concentrations
- › **Fecal samples (56):** all negative
- › **Soil samples (220):** 6 doubtful positives (ZnCl₂ flotation and qPCR)
- › **Camera traps** >>





Camera traps

- › Foxes detected on **6 / 14** farms
- › Seem to deliberately enter farm plots to forage fruits







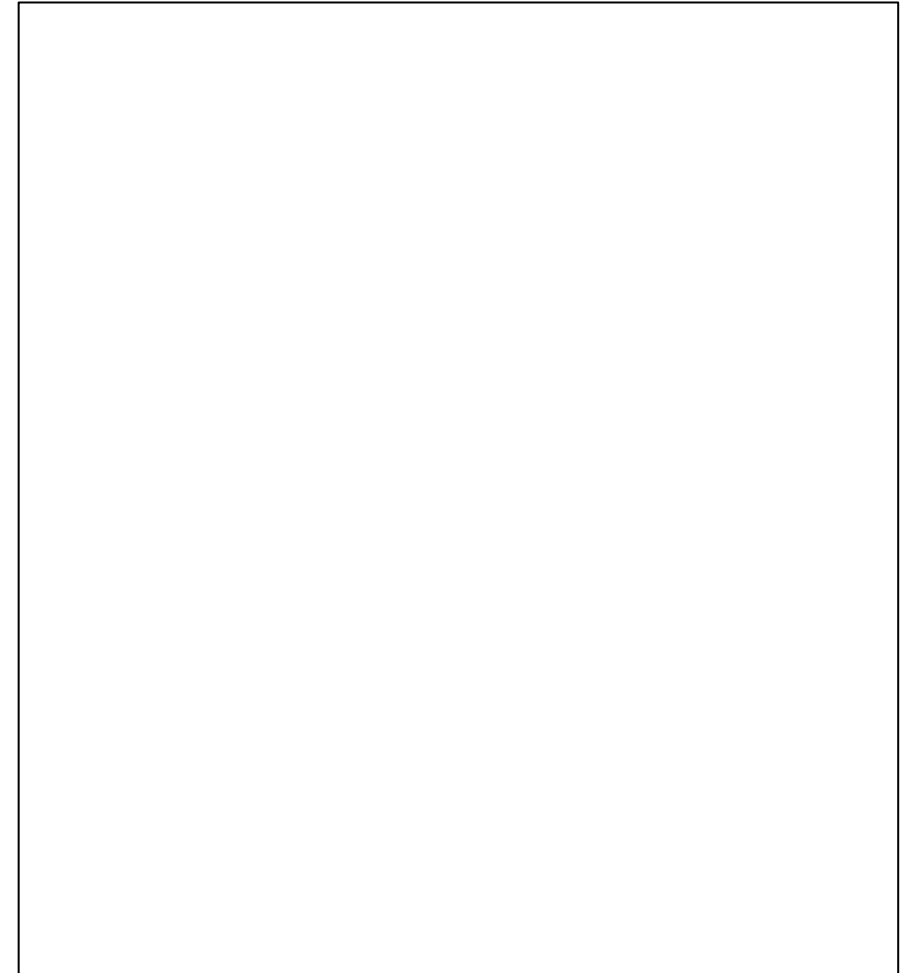
Interpretation of results

- › **Confirmation** of potential *E. multilocularis* contamination (2/14 > 4.5%)
 - › **Confirmation** of presence of foxes on fruit farms
 - › Low [DNA] and only some positive samples confirmed by sequencing (3/10)
 - › Positive samples from central Limburg
-
- › Infectious or non-infectious eggs?
 - › Extension of the endemic area to central Limburg?
 - › Risk factor analysis still to be done



Fox study (2024-2026)

- › Foxes collected by hunters
 - 75 participating hunters
- › Weekly dissections and sample collection
 - Colon content + fur → Em test
- › Goal: 225 foxes over 2 winters





Foxes: results so far

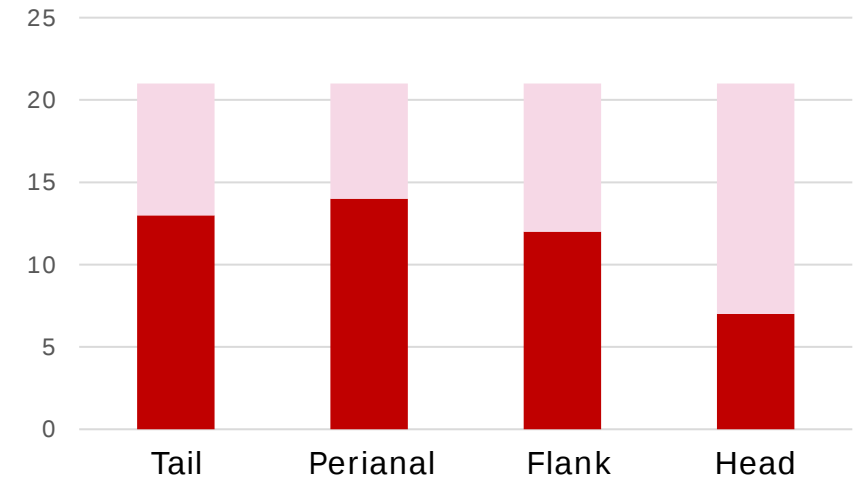
- › >70 hunters and 128 foxes
- › *E. multilocularis* qPCR (5x colon content)
 - **21 / 128 positives** (16%)
 - Only South Limburg (local prevalence: 26%)
- › Sedimentation & Counting Technique (4 segments)
 - All qPCR positive samples, and sample of negatives
 - Worm burden **8 – 20.000**





Foxes: results so far

- › Positive results fur
 - Positive (SCT/qPCR) foxes
 - Wash-Centrifuge-Extract DNA-qPCR



Foxes: to be done

- › 2025-2026
 - Minimum 100 foxes
 - Extend study area more to the North
- › Data analysis
 - Distribution, prevalence and load, modelling risk of h





Extra: gorilla AE case april-2024

- › 25-year-old Western lowland gorilla
- › Born in England, transfer to Limburg in 2013
- › Symptoms after giving birth
 - Lethargic, anorexic, neurological symptoms
- › Unsuccessful treatments
- › Ultrasonography: abces-like liver lesions
- › Serology: *Echinococcus multilocularis*
- › Pathology FVM, qPCR RIVM
 - **Diagnosis:** alveolar echinococcosis





Extra: gorilla case

- › Zoo visit in August 2024: food
- › Fox feces and soil samples: negative
- › Additional serological testing
 - Case was negative in 2016
 - No other positives found ... but not all have been tested (anesthesia)
- › First case of AE in a Dutch zoo animal
 - Reported in gorillas in Switzerland (2003) and Japan (1995)
- › Pregnancy: disease progression and challenges newborn
- › Public health implications





Summary



E. multilocularis is still a public health risk in Limburg



Potential contamination of outdoor grown fruits and other crops



No spread to Central Limburg observed in foxes (yet)



Gorilla case demonstrates risk of susceptible species



Thank you!



Laura Derks (PhD candidate)

Supervising team

Joke van der Giessen
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Andrea Gröne (UU)

Dissection team

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Lab & support team

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Helga Waap
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External partners

All fruit producers, fruit organisations,
hunters, KNJV, NVWA, zoo employees, ...