Experience with alternative tests to diagnose *Echinococcus multilocularis* infection in definitive hosts

Which diagnostic method is best suited for epidemiological studies?

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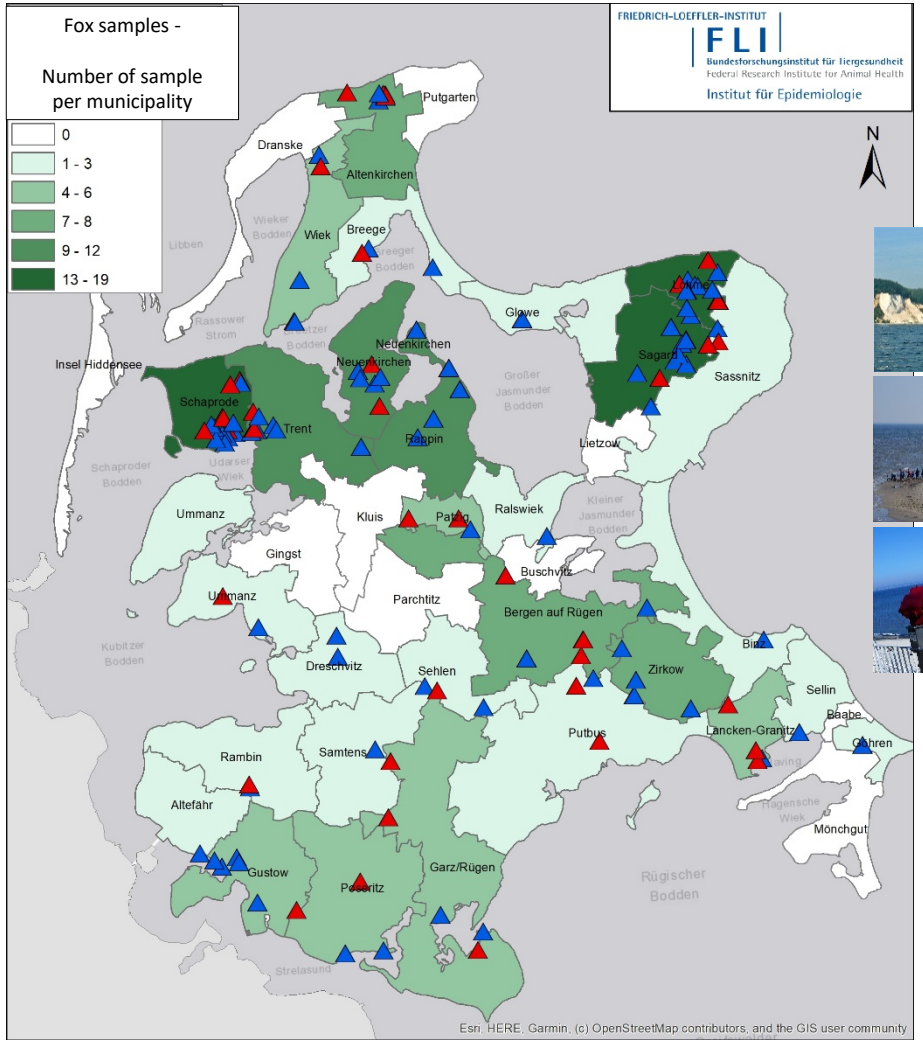
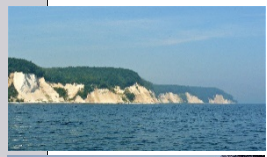
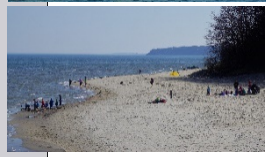
Background – Study on *Echinococcus multilocularis* on the island Rügen (Touristic hotspot)

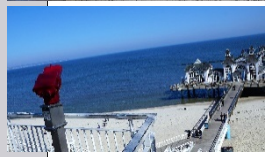
More than 400 carcasses collected by hunters from Nov. 2023 until Nov. 2024

→ 29% of foxes positive
(47/162 assessed until 16. Sept. 2024)

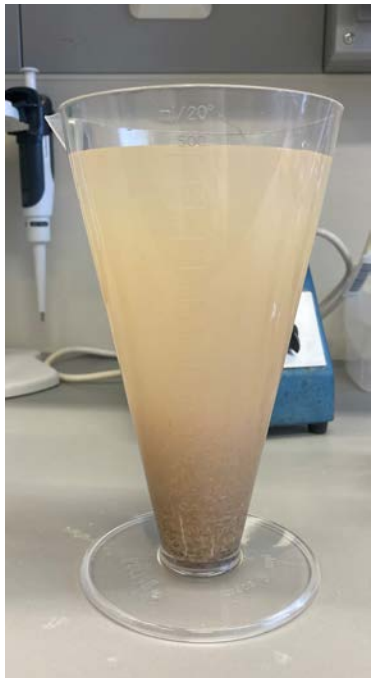








Sedimentation and Counting Technique (SCT) is laborious and not efficient



SCT is regarded as a reference method, although limitations of this technique are known and were reported, previously.

- Limited diagnostic sensitivity relative to PCR-based methods is evident
- Time consuming
- Experienced personnell is necessary to microscopically inspect and count

Alternative methods?

Various sample types were tested

Alternative methods?

- PCR-based method
- Samples should be easy to collect and to store
- Minimal storage capacity needed

One of our technicians, Alrik Kunisch, proposed swabbing

Using swabs? – Two swabbing methods were tested

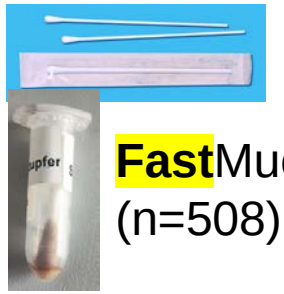
Using fecal samples? – As already shown by others

Using **SCT** – As a reference (n=508)

Samples from
- 399 foxes
- 109 racoon dogs

Inactivated at -80 °C, 7 days

IsolMucSwab (n=403)



FastMucSwab (n=508)

Feces (n=105)

DNA-Extraction

DNA-Extraction

Em-qPCR, Primers and MGB probe (Isaksson et al. 2016);
Positive < Ct 39
Inconclusive 39-41
Negative > Ct 41

Comparison of two swabbing methods – FastMucSwab *vs* IsolMucSwab

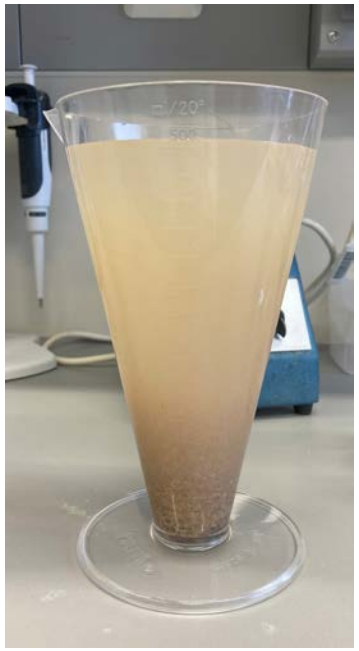
Superior in terms of convenience

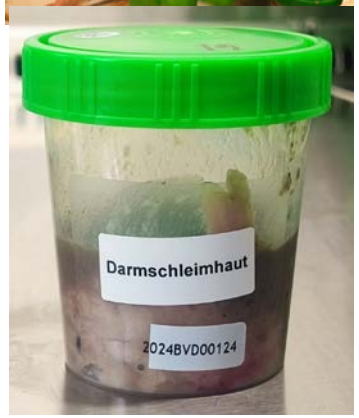
SCT

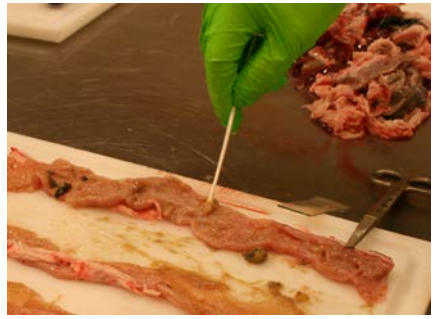
IsolMucSwab Em-qPCR	FastMucSwab Em-qPCR		
Total, n=403	**Positive**	**Incon-clusive**	**Negative**
Positive	**60**	0	6
Inconclusive	0	0	3
Negative	7	4	**323**

Agreement characterized by a ***kappa*** coefficient **0.86** (95% CI: 0.79–0.92; almost perfect).

Comparison of FastMucSwab relative to SCT as a reference







Advantages:
- Easier
- Less time consuming
- Needs less storing capacities

SCT result	FastMucSwab Em-qPCR		
Total, n=508	**Positive**	**Inconclusive**	**Negative**
SCT positive	**63**	0	5
SCT negative	37	4	**399**

Diagn. **sensitivity**: **92.7%** (95% CI: 83.0–97.3%)
Diagn. **specificity**: **91.5%** (95% CI: 88.4–93.9%)

Agreement characterized by a ***kappa*** coefficient **0.70** (95% CI: 0.62–0.79; substantial).

2, 3, 8, 27, and 75 worms in SCT

FastMucSwab Em-qPCR results reflect quantitative SCT findings

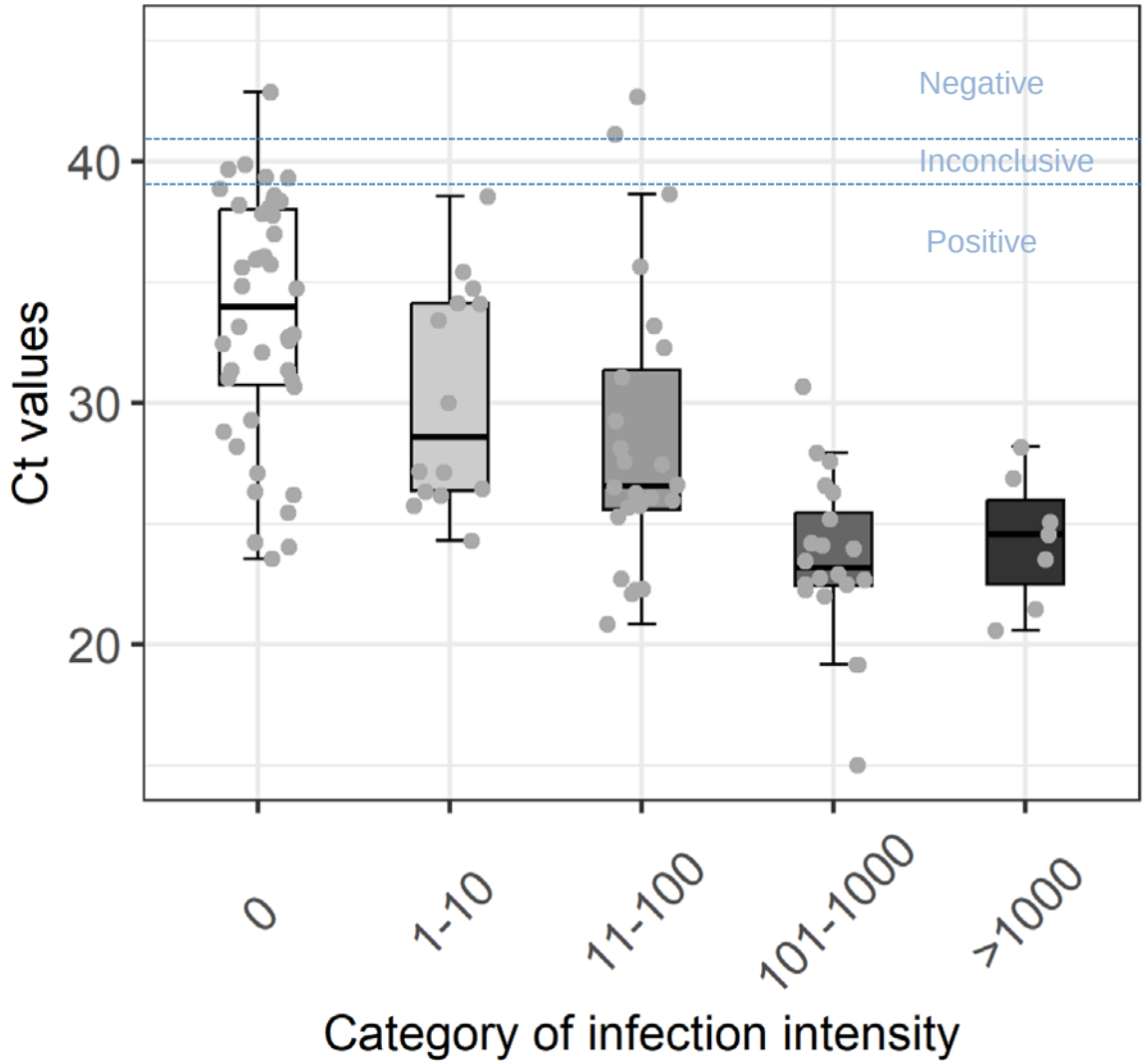
Ct values were stratified for the number of *E. multilocularis* worms in SCT

All samples with a Ct value (n=107)

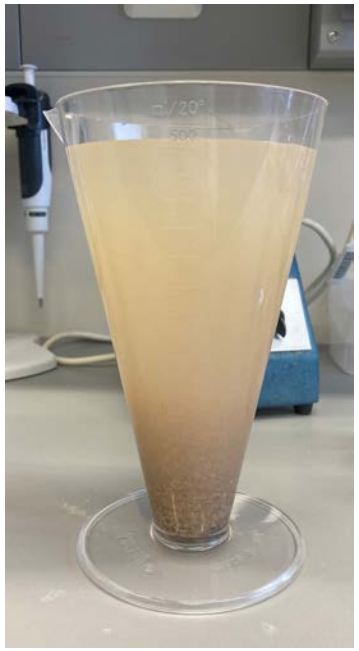
SCT categories

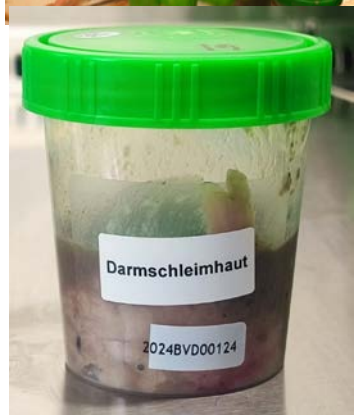
- >1000 worms (n=7 animals)
- 101–1000 worms (n=20 animals)
- 11–100 worms (n=24 animals)
- 1–10 worms (n=14 animals)
- 0 worms (n=42 animals)

There is a clear relationship, although weak



Comparison of SCT *vs.* fecal samples





SCT	Feces Em-qPCR			
Total, n=105	**Positive**	**Inconclusive**	**Negative**	Diagn. **sensitivity**: **82.8%** (95% CI: 63.5–93.5%)
SCT positive	**24**	0	5	Diagn. **specificity**: **90.9%** (95% CI: 81.4–95.9%)
SCT negative	7	0	**60**	

Agreement characterized by a ***kappa*** coefficient **0.72** (95% CI: 0.57–0.87; substantial).

1, 3, 3, 6, and 13 worms in SCT

Comparison of EM-qPCR results in feces *vs* FastMucSwab, stratified by SCT result

	Feces Em-qPCR	FastMucSwab Em-qPCR		
SCT positive and negative (n=105)		Positive	Inconclusive	Negative
	Positive	26	0	7
	Inconclusive	0	0	0
	Negative	5	0	67
SCT positive (n=29)				
	Positive	21	0	4
	Inconclusive	0	0	0
	Negative	3	0	1
SCT negative (n=76)				
	Positive	5	0	3
	Inconclusive	0	0	0
	Negative	2	0	66

Comparison of EM-qPCR results in feces *vs* FastMucSwab, stratified by SCT result

	Feces Em-qPCR	FastMucSwab Em-qPCR	
SCT positive and negative (n=105)		Positive	Negative
	Positive	26	7
	Negative	5	67
SCT positive (n=29)			
	Positive	21	4
	Negative	3	1
SCT negative (n=76)			
	Positive	5	3
	Negative	2	66

3 worms in SCT

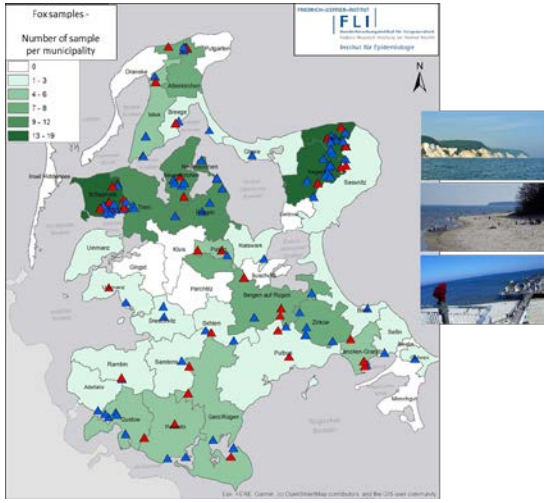
A combination of Em-qPCR results obtained on feces and on FastMucSwab reached highest diagnostic sensitivity (i.e. only 1 false negative)

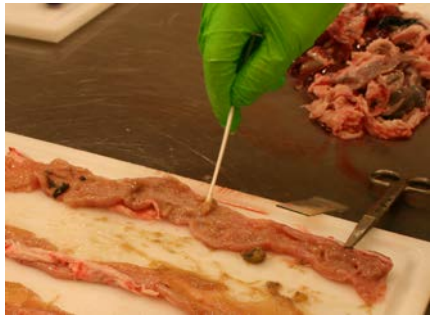
Summary

- As a simple alternative method the FastMucSwab Em-qPCR seems to represent an efficient tool for performing larger-scale epidemiological studies

- FastMucSwab Em-qPCR performed very similar to Em-qPCR based on fecal DNA in detecting SCT positive samples

- In both these qPCR-based methods, a number of SCT negative samples tested positive, which – similar to previous studies - suggests limitations regarding diagnostic sensitivity of SCT





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Chemisches und Veterinäruntersuchungsamt Westfalen

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Thank you very much for your attention!




Comparison of two swabbing methods – FastMucSwab *vs* IsolMucSwab

Superior in terms of convenience

SCT

IsolMucSwab Em-qPCR	FastMucSwab Em-qPCR		
Total, n=403	**Positive**	**Incon-clusive**	**Negative**
Positive	**60**	0	6
Inconclusive	0	0	3
Negative	7	4	**323**

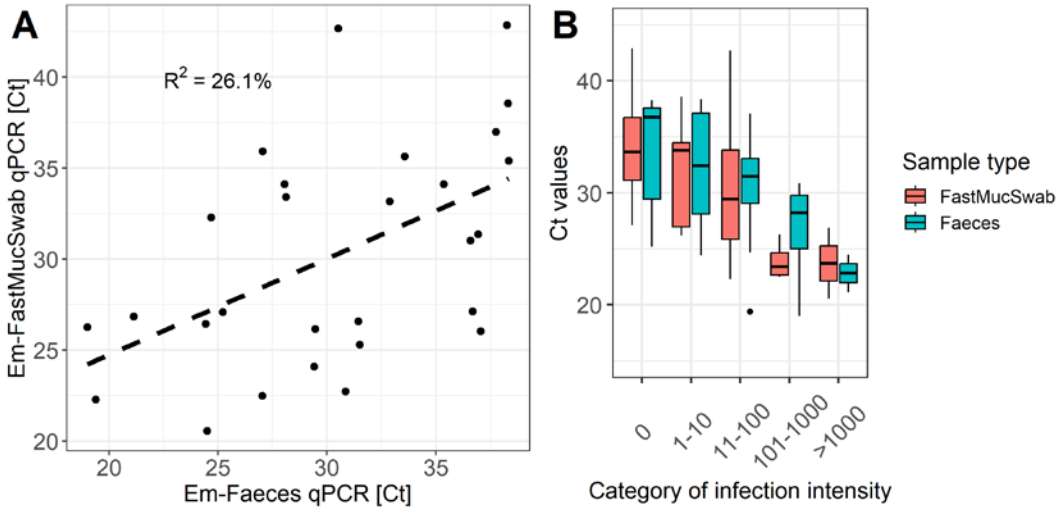
0, 0, 277, 0, 0, 0 worms in SCT

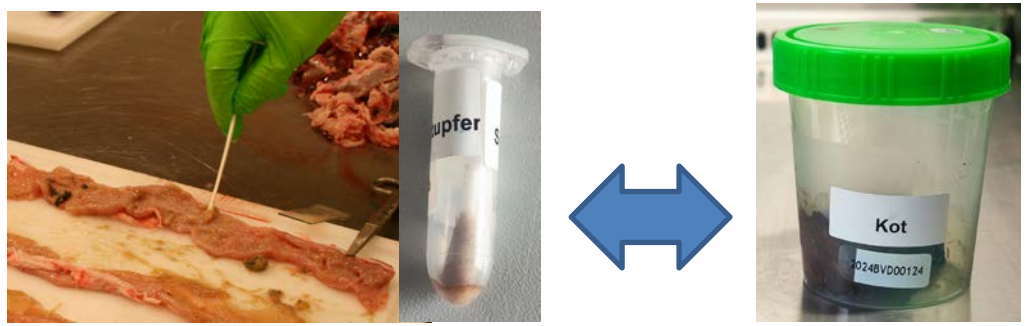
0, 0, 0, 75, 0, 0, 0 worms in SCT

Agreement characterized by a ***kappa*** coefficient **0.86** (95% CI: 0.79–0.92; almost perfect).

Comparison of FastMucSwab *vs* feces as a sample matrix.

Ct values in FastMucSwab Em-qPCR are correlated with the Ct values observed by Feces Em-qPCR.
(A) Linear regression (dashed line)
(B) Both Em-PCR results show decreasing median Ct values with increasing numbers of *E. multilocularis* worms, as determined by the sedimentation counting technique (SCT).
SCT results, displayed as infection intensity, were categorized as follows:
> 1000 (n=2 animals),
101-1000 (n=4 animals),
11-100 (n=8 animals,
1-10 (n=8 animals)
0 (no worms detected, n=6 animals).





Project on Germanies largest island

8 sites with -20 °C freezers
on the Island + 10 km radius