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Denmark: *Echinococcus multilocularis* in wildlife - surveillance programs and diagnostic tools

Rosalina Rotovnik, DVM // roro@ssi.dk
Danish Society for Parasitology & Scandinavian-Baltic Society for Parasitology

Lifecycle & Disease – *Echinococcus multilocularis*

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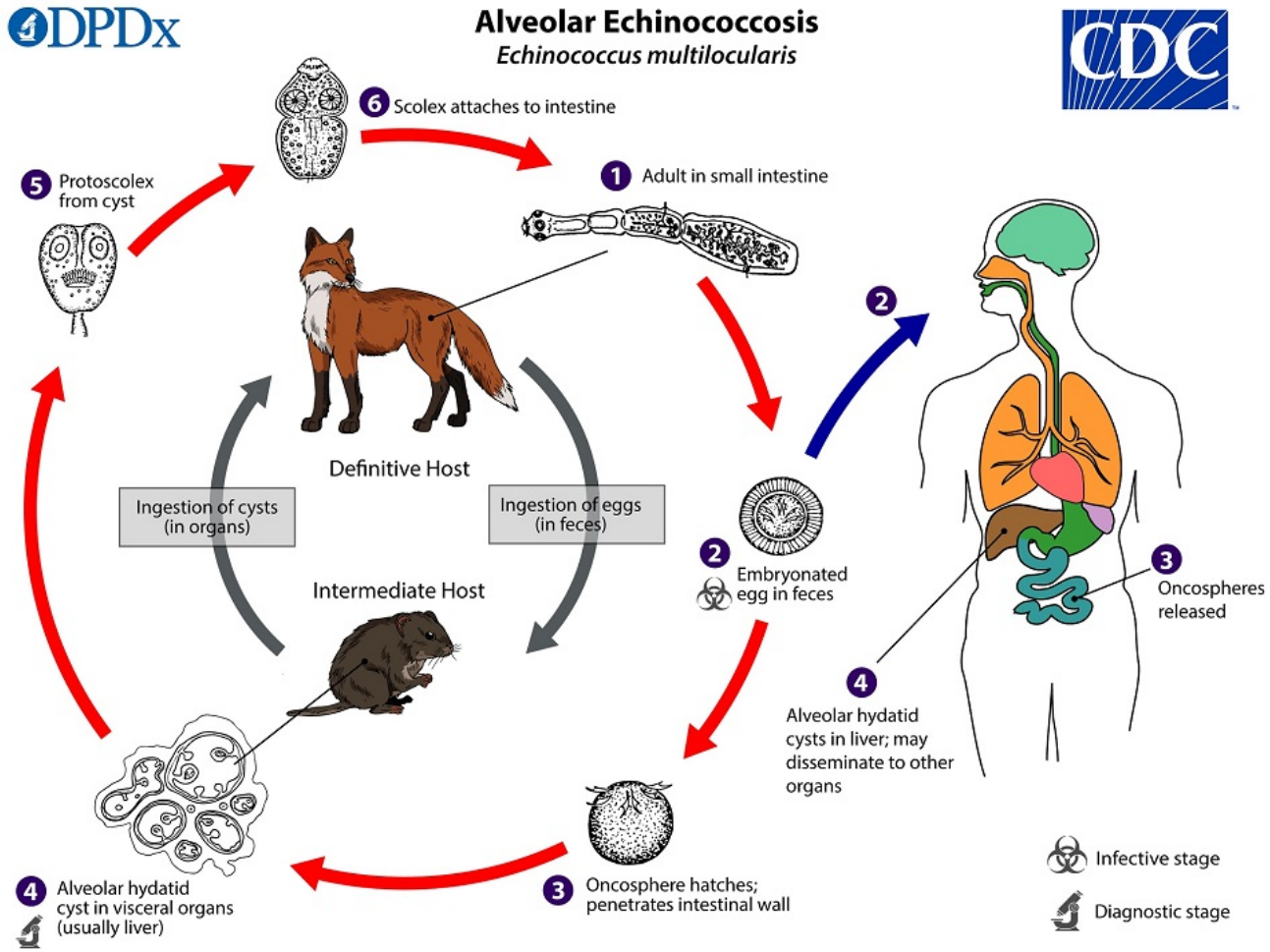
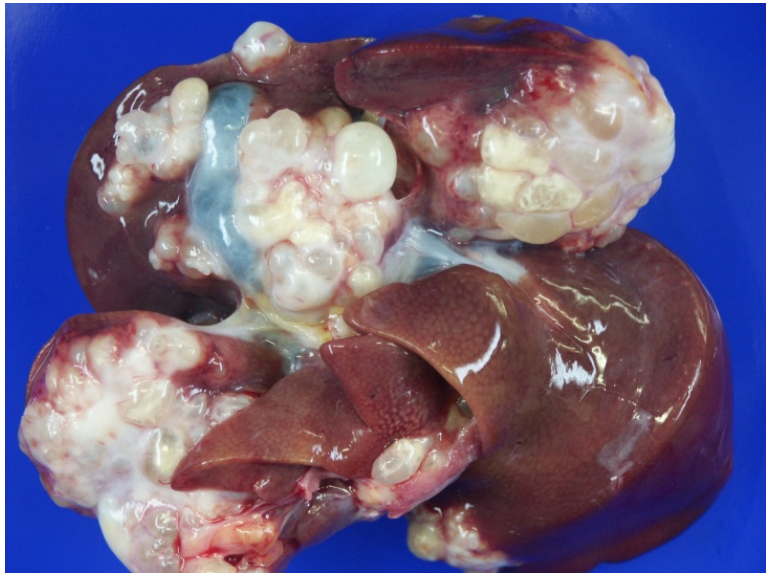


https://www.cdc.gov/dpdx/echinococcosis/index.html

Alveolar echinococcosis, liver



https://www.dpz.eu/en/unit/pathology/research/pathology-of-non-human-primates/echinococcosis.html

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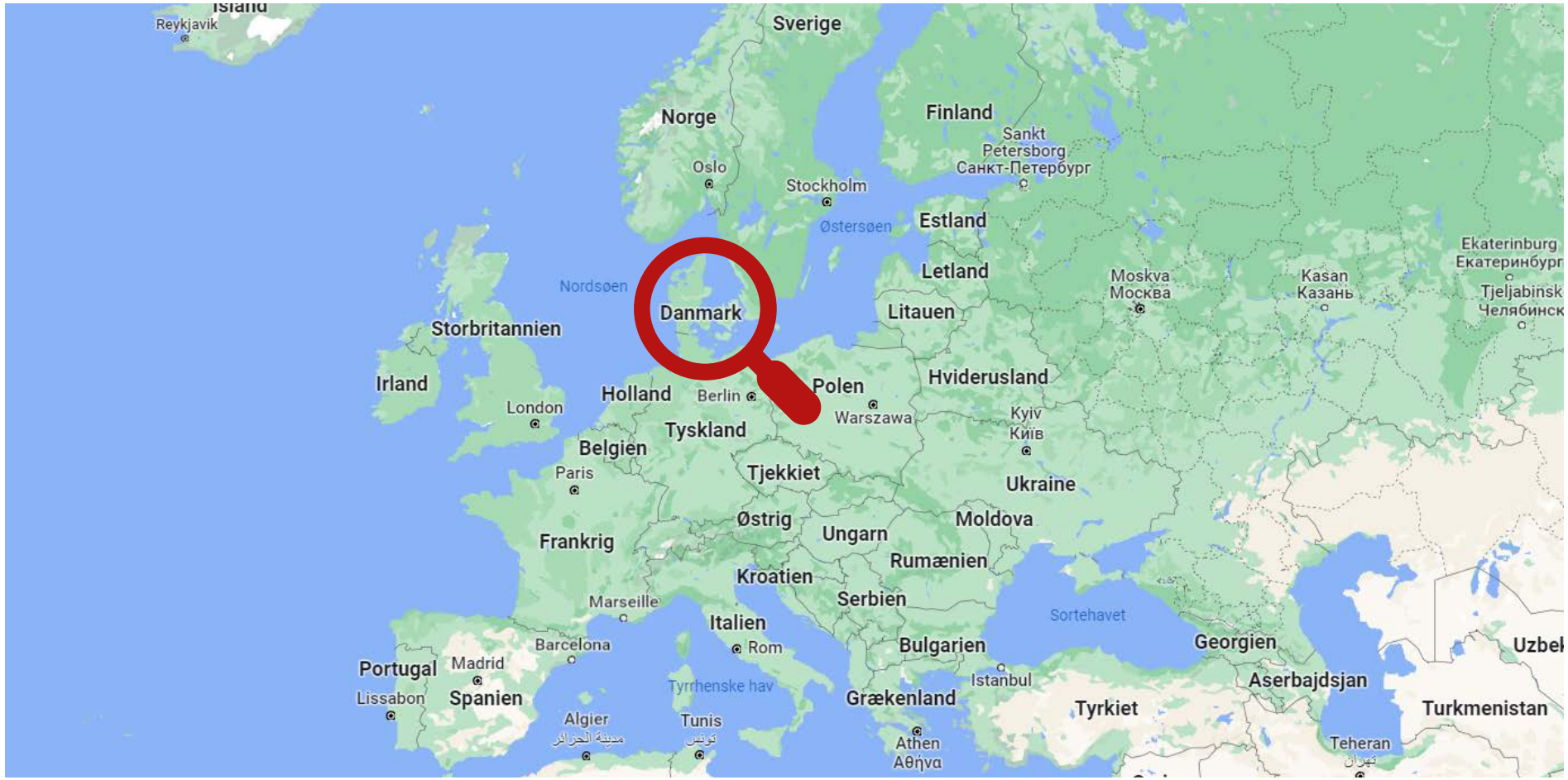

Echinococcus multilocularis in Denmark

Denmark – Few human cases of echinococcosis

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- Very few cases of echinococcosis in humans, and so far no autochthonous infections
- No systematic surveillance

UGESKR LÆGER 166/10 | 1. MARTS 2004

VIDENSKAB OG PRAKSIS | KASUISTIK

Echinococcus multilocularis hos en dansker

Afdelingslæge Alex Lund Laursen & reservelæge Kim P. David

H:S Rigshospitalet, Epidemiklinikken

med relativ hyppig spredning til andre organsystemer. Udviklingstiden fra smitte til symptomdebut er som oftest lang, sædvanligvis mere end ti år. Debutsymptomerne kan være vage øvre abdominalsmerter, om end de fleste patienter debuterer

Journal of TRAVEL MEDICINE

Cystic Echinococcosis of the Liver: Experience From a Danish Tertiary Reference Center (2002–2010)

Sonia Branci, MD,* Caroline Ewertsen, MD, PhD,* Søren Thybo, MD,† Henrik V. Nielsen, PhD,‡ Flemming Jensen, MD,* André Wettergren, MD, DMSc,§ Peter N. Larsen, MD,§ and Ib C. Bygbjerg, MD, DMSc†‖

Departments of *Radiology and †Infectious Diseases, Rigshospitalet, Copenhagen OE, Denmark; ‡Department of Parasitology and Mycology, Statens Serum Institut, Copenhagen S, Denmark; §Department of Gastrointestinal Surgery, Rigshospitalet, Copenhagen OE, Denmark; ‖Faculty of Health Sciences, University of Copenhagen, Copenhagen K, Denmark

DOI: 10.1111/j.1708-8305.2011.00577.x

ELSEVIER

Contents lists available at ScienceDirect

IDCases

journal homepage: www.elsevier.com/locate/idcases

Case report

Pulmonary cystic echinococcosis acquired during a short-term tourist travel

Siri Nana Halling Svensgaard [a,*], Pikka Jokelainen [b], Christen Rune Stensvold [c], Karen Rokkedal Lausch [a], Anette Højsgaard [d], Johanne Lade Keller [e], Henrik Vedel Nielsen [c], Carsten Schade Larsen [a]

[a] Department of Infectious Diseases, Aarhus University Hospital, Denmark
[b] Infectious Disease Preparedness, Statens Serum Institut, Copenhagen, Denmark
[c] Department of Bacteria, Parasites and Fungi, Statens Serum Institut, Copenhagen, Denmark
[d] Department of Cardiothoracic and Vascular Surgery, Aarhus University Hospital, Denmark
[e] Department of Pathology, Aarhus University Hospital, Denmark

Denmark – DNA in the Environment

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- *E. multilocularis* DNA on fresh food sources in Denmark

  - OHEJP Meme Multicentre study for detection of Em, Eg sl (and other taenids), found on <u>berries and salad</u>

One HEALTH EJP MeME

The Project #MEME

Start:	1 January 2020
Duration:	3.0 Years
Domain:	Emerging Threats
Keywords:	Echinococcus spp., cystic and alveolar echinococcosis, helminth parasites, molecular epidemiology, zoonotic helminths
Contact:	Adriano Casulli (ISS)

Denmark – Surveillance in wildlife

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1997-2002

1040 foxes, three positive

- Local foci near Copenhagen

2009-2012

483 foxes & raccoon dogs, one positive fox

- Increasing occurrence in Germany and Sweden

2012-2015

1345 foxes & raccoon dogs,

19 foxes and two raccoon dogs positive

- High local prevalence in South Jutland

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Available online at www.sciencedirect.com
SCIENCE DIRECT

Veterinary Parasitology 139 (2006) 168–179

veterinary parasitology

www.elsevier.com/locate/vetpar

Helminths of red foxes (*Vulpes vulpes*) in Denmark

I. Saeed [a,*], C. Maddox-Hyttel [b], J. Monrad [a], C.M.O. Kapel [a]

[a] *Danish Centre for Experimental Parasitology, Department of Veterinary Pathobiology, The Royal Veterinary and Agricultural University, Dyrlaegevej 100, DK-1870 Frederiksberg C, Denmark*
[b] *Section for Immunology and Parasitology, Danish Institute for Food and Veterinary Research, Bülowsvej 27, DK-1790 Copenhagen V, Denmark*

Received 13 June 2005; received in revised form 6 February 2006; accepted 14 February 2006

International Journal for Parasitology: Parasites and Wildlife 2 (2013) 144–151

ELSEVIER

Contents lists available at SciVerse ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Endoparasites of the raccoon dog (*Nyctereutes procyonoides*) and the red fox (*Vulpes vulpes*) in Denmark 2009–2012 – A comparative study

Mohammad Nafi Solaiman Al-Sabi [a,*], Mariann Chriél [a], Trine Hammer Jensen [b], Heidi Larsen Enemark [a]

[a] *Section for Bacteriology, Pathology and Parasitology, National Veterinary Institute, Technical University of Denmark, DK-1870 Frederiksberg C, Denmark*
[b] *Department of Biotechnology, Chemistry and Environmental Engineering Aalborg University/Aalborg Zoo, DK-9000 Aalborg, Denmark*

Parasitology Research (2018) 117:2577–2584
https://doi.org/10.1007/s00436-018-5947-y

ORIGINAL PAPER

Echinococcus multilocularis in Denmark 2012–2015: high local prevalence in red foxes

H. H. Petersen [1] · M. N. S. Al-Sabi [1,2] · H. L. Enemark [1,3] · C. M. O. Kapel [4] · J. A. Jørgensen [4] · M. Chriél [1]

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Denmark – Surveillance in wildlife

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Received: 5 March 2018 / Accepted: 24 May 2018 / Published online: 1 June 2018
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2012-2015

1345 foxes & raccoon dogs,

19 foxes and two raccoon dogs positive

- High local prevalence in South Jutland

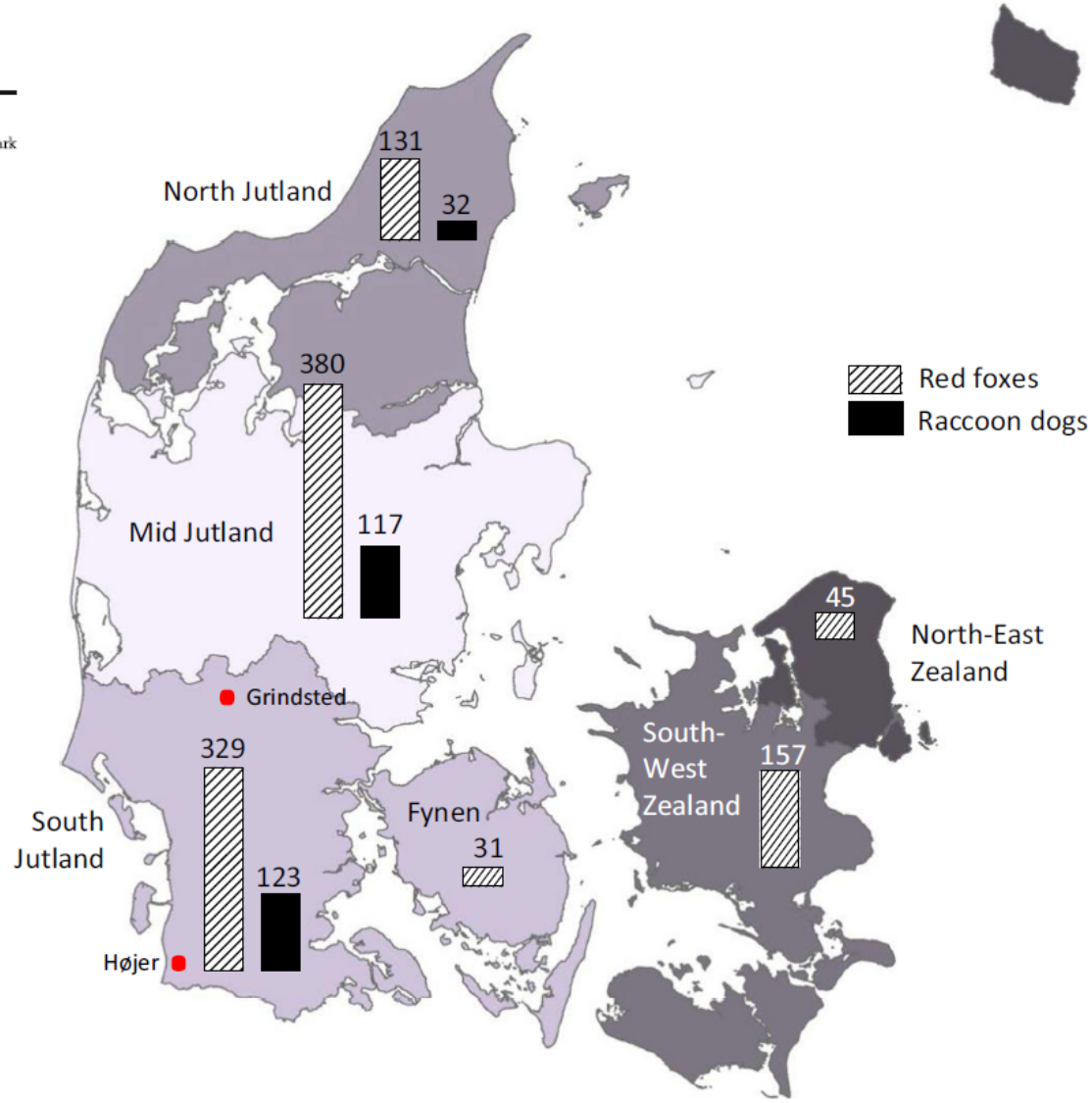

Fig. 1 Map of Denmark illustrating the number of red foxes and raccoon dogs examined for *Echinococcus multilocularis* per region

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Denmark 2022:
Governmental wish for new surveillance project

Advances in diagnostic tools and genetic information
+ New veterinary disease preparedness structure

-> New diagnostic tools

Echinococcus spp. diagnostics at SSI

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Human

Serology

PCR + genotyping of cyst material

Veterinary

Faecal sedimentation of taeniid eggs

PCR analysis of fecal samples

- (Two different taeniid PCR primer sets)

Sedimentation and counting (intestines)

Development of Real-time PCR

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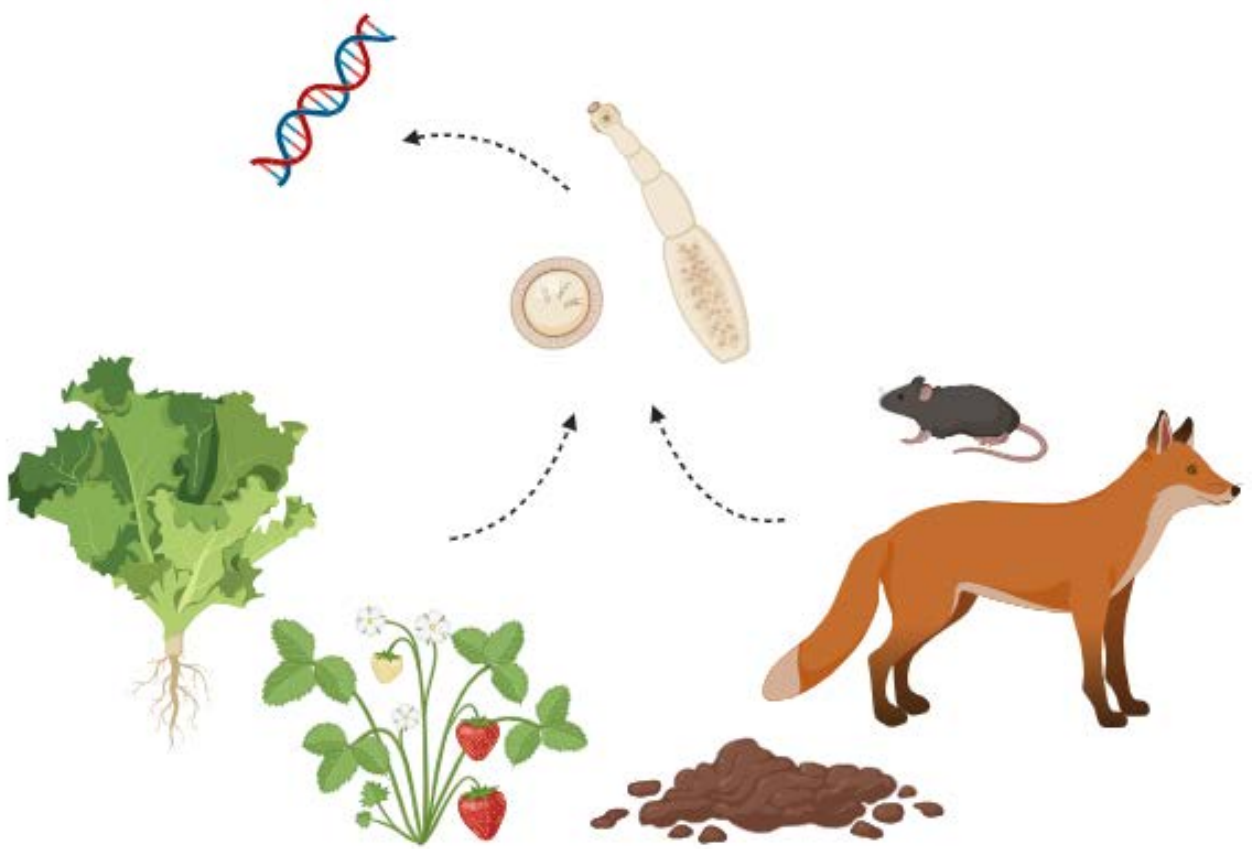
- Aim: To detect even small amounts of *E. multilocularis* DNA in intermediate hosts, fecal- and environmental samples
  (High sensitivity needed!)

- In collaboration with:
  Dr Gérald Umhang,
  Head of National Reference Laboratory for *Echinococcus* spp. at Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (ANSES), Malzéville, Frankrig



Created in BioRender.com bio

Development of Real-time PCR

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- Process:
  - Mitochondrial genome sequences representing *E. multilocularis* aligned and visualized
  - Identification of areas for primers and probes
- Tested on:
  - DNA from E.m., E.g.s.s. (G1-G3) and E.g.s.l. (G4; G5; G7; G10) – Dr Gérald Umhang
  - Fox faecal samples spiked with E.m.
  - DNA from other helminths for crossreaction testing
- Result: real-time PCR for *E. multilocularis* with high sensitivity and high specificity
  - Sensitivity: 5 eggs pr. Gram
  - Specificity: *E. granulosus* & other helminths not detected / Few with Ct >40

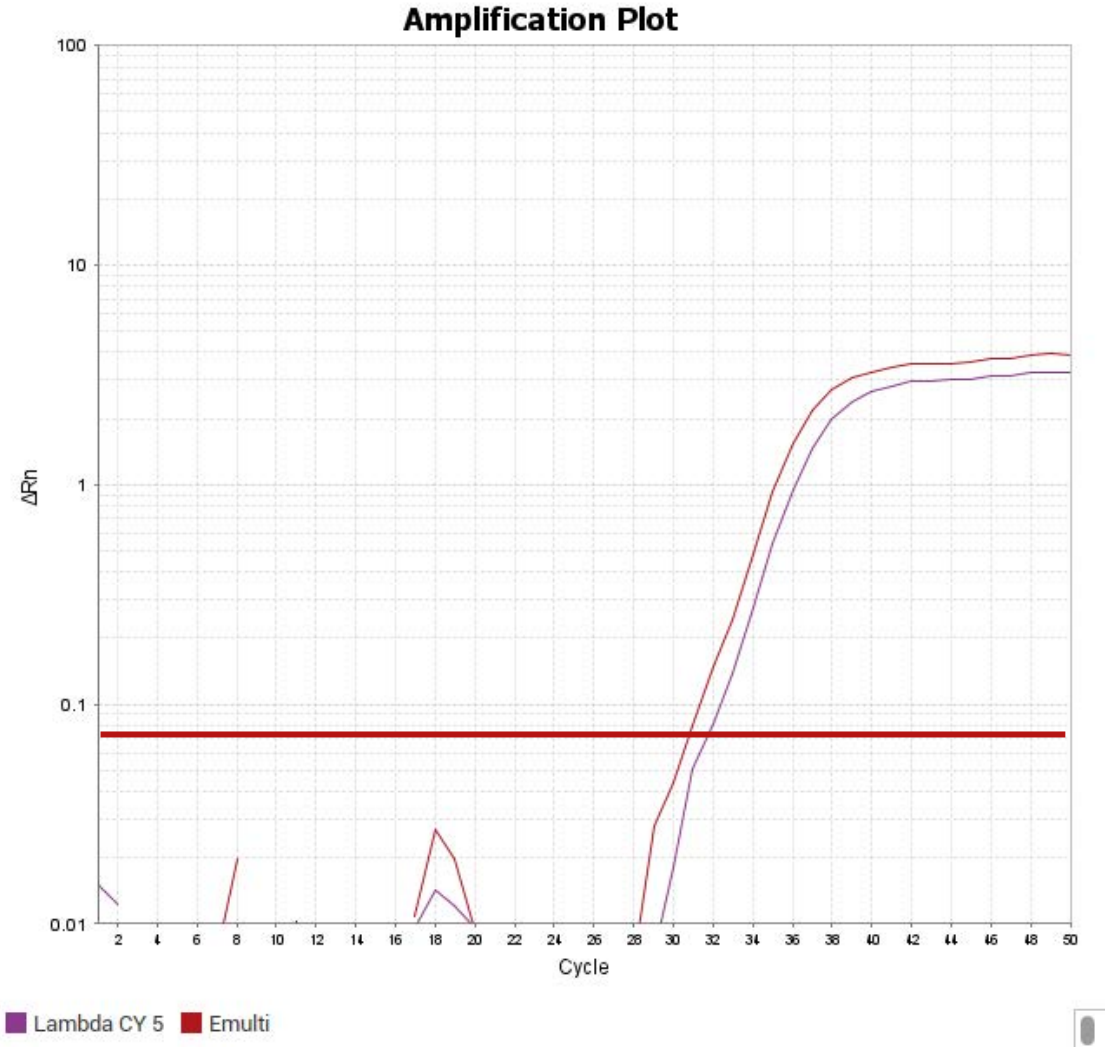
Set-up

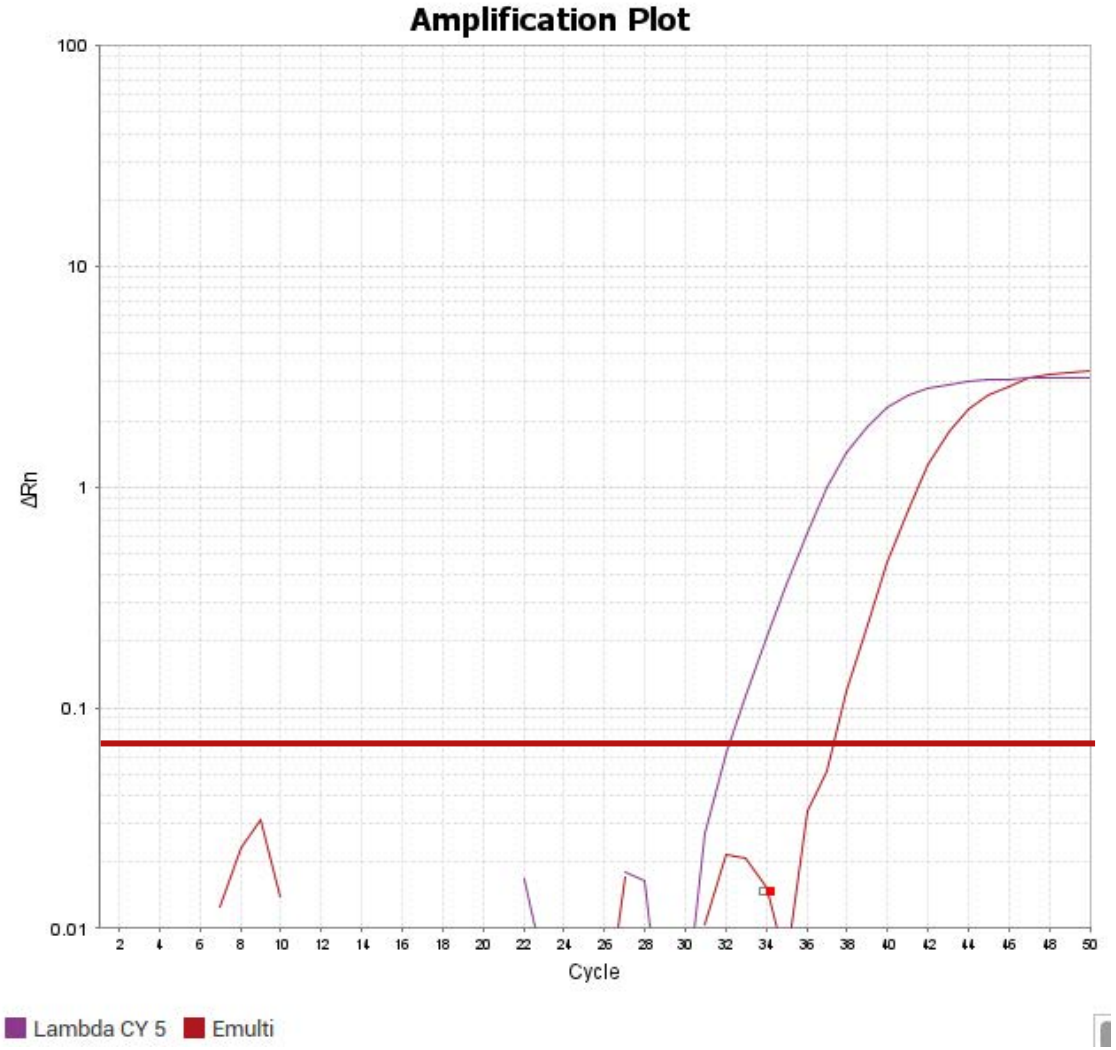
- Target: 12S mitochondrial DNA
- Prod. size: 119 bp
- Primers: 1 F and 1 R
- Probe: 1 (E.m.)
- Inhibition control (Lambda)

Positive controls

Dilution 10^{-2}: CT<31 and 10^{-4}: CT<38

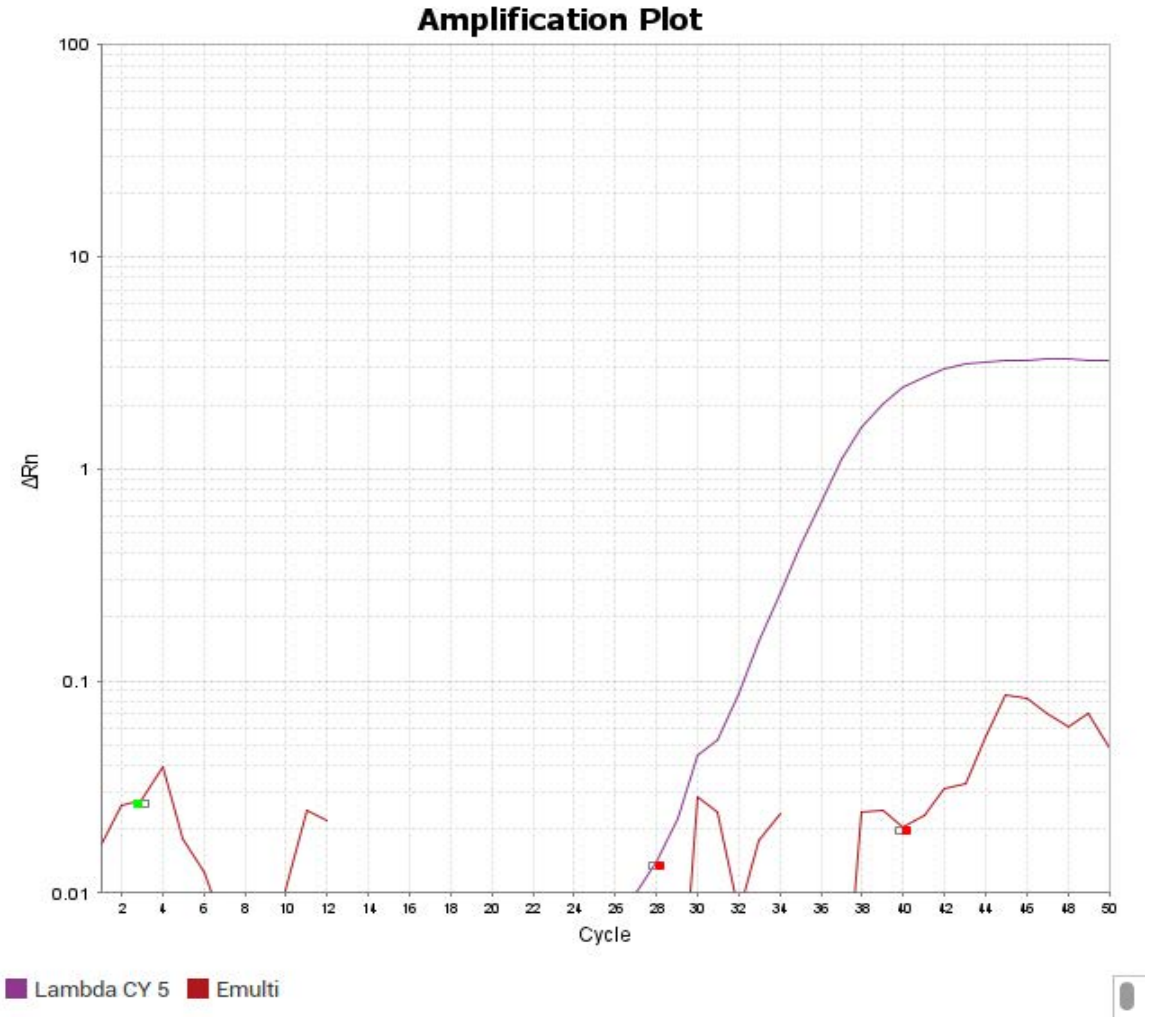
Cut-off: CT>40





Quant Studio, Thermo Fischer Scientific

Negative control



Quant Studio, Thermo Fischer Scientific

People Involved in Development of Real-Time PCR

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- Christen Rune Stensvold: Test design, gathering of test material, validation, communication with ANSES expert Gérald Umhang and formulating the validation report
- Jørgen Skov Jensen: Test design
- Rebecca Berg & Rosalina Rotovnik: Litterature search and veterinary disease preparedness aspects, implementation in surveillance projects and validation report proof-reading
- Gitte Jensen: Technical validation
- Henrik Vedel Nielsen & Randi Føns Petersen: Continuous operation, maintenance and quality assurance

- The analyses has been developed in collaboration with Dr Gérald Umhang, Head of National Reference Laboratory for *Echinococcus* spp. at Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail (ANSES), Malzéville, Frankrig. His expertise and provision of sample panels has been indispensable.

Current surveillance

- **300 foxes in 2022-2023**
  - SSI and the University of Copenhagen (Danish Veterinary Consortium – DK-VET)
  - Governmental financing

Pathology watch, University of Copenhagen

- **200 small predators and scavengers in 2023-2024**
  - SSI and Aarhus University – Project ”Mårdyrsundhed” (marten species health)
  - External financing

https://skadedyrshop.dk/andre-dyr/mar-mink-ilder-loaekat

- **Human cases**
  - New official governmental announcement (27-10-2023) *E. multilocularis* and *E. granulosus* now reportable to SSI – positive samples, submission mandatory!

E. multilocularis in foxes 2023 (DK-VET)

UNIVERSITY OF COPENHAGEN

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- 300 foxes in 2022-2023
  - SSI and the University of Copenhagen (UCPH) / Danish Veterinary Consortium (DK-VET)
  - Governmental financing / Danish Veterinary and Food Administration (FVST)

Pathology watch, University of Copenhagen

Procedure:

Fox necropsy Pathology watch UCPH → Magnetic Capture DNA extraction → *E. Multilocularis* Real-time PCR → Positive results reported to UCPH and FVST

Wormcount by SCT Parasitology, UCPH → Positives confirmed by PCR and Sanger → Final report with UCPH → Report to FVST every 6 months

E. multilocularis in foxes 2023 (DK-VET)

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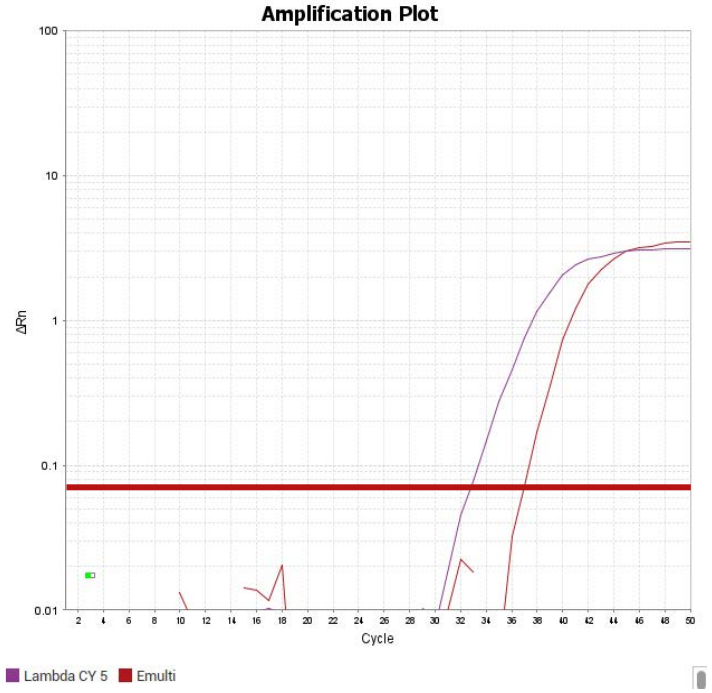
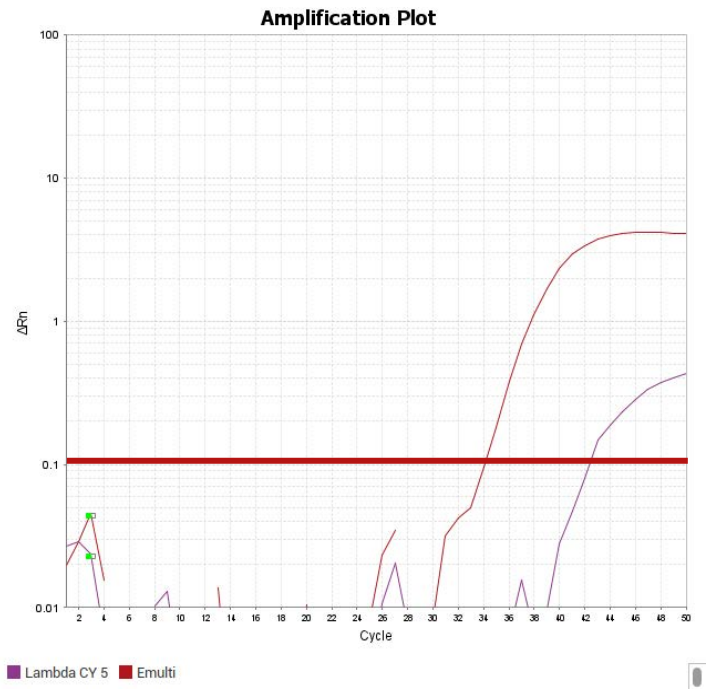
Results (unpublished)

- 149 samples / 3 positives - from Southern Jutland (bordering Germany)



CT: 37



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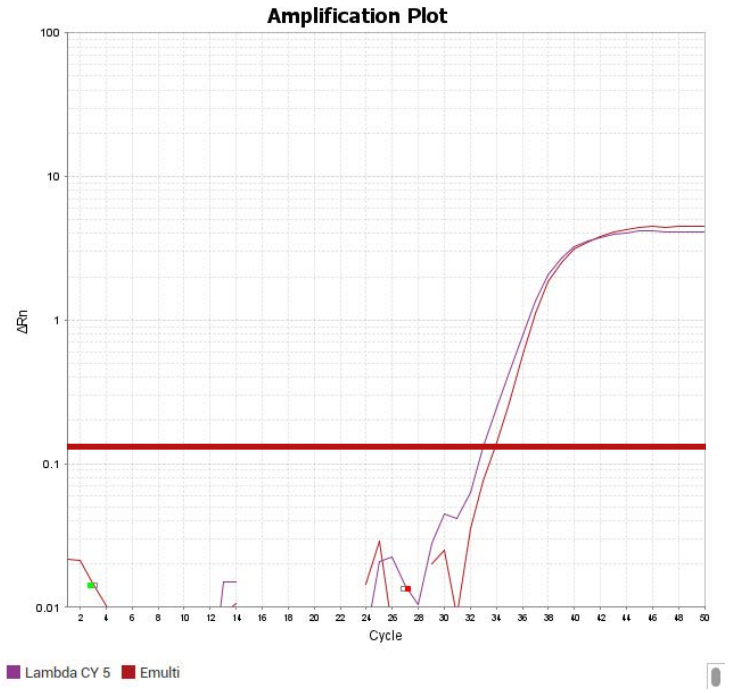

CT: 34

Quant Studio, Thermo Fischer Scientific

E. multilocularis in foxes 2023 (DK-VET)

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Confirmation

- 3/149 positive samples - from Southern Jutland

In house taeniid PCR

Nr	DNA		Nr	DNA	
1	ER 93	V+			
2	ER 93	1:10 V			
3	ER 110	(V)+			
4	ER 110	1:10			
5	ER 114	V+			
6	ER 114	1:10 (V)			
7	KONTROL	1:10 V			
8	H_2O				

Sedimentation and counting
- mixed taeniid infection in ER-93!
Parasitology department, UCPH

Sanger by Eurofins (fecal DNA)

SSI ID	Sanger result
ER-93	*Taenia polyacantha*
ER-110	*E. multilocularis*
ER-114	*E. multilocularis*

Sanger by Eurofins (worm DNA)

SSI ID	Sanger result
ER-93	*E. multilocularis*
ER-110	*E. multilocularis*
ER-114	*E. multilocularis*

Take home message

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- Few human cases of echinococcosis in Denmark, and most focus is on cystic echinococcosis
- A sensitive real-time PCR was developed and validated in-house at SSI in collaboration with Dr Gérald Umhang (ANSES)
- *Echinococcus multilocularis* is still present in wildlife in Denmark with recent findings in the south
- Fruitfull collaboration: Danish Veterinary Consortium (DK-VET)
  - University of Copenhagen and Statens Serum Institut

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- Sussie Pagh

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Thank you