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# First report of *Taenia ovis* infection in Danish sheep (*Ovis aries*).

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## Graphical abstract

## Highlights

- We identified the metocestode stage of *Taenia ovis* from domestic sheep in Denmark
- This is the first report of *Taenia ovis* infection in Danish sheep
- The Danish isolate was aggregated in one branch together with isolates from Asia
- This parasite was probably introduced to the farm by recently imported dogs

## Abstract

We report *Taenia ovis* infection in Danish sheep for the first time. In spring 2016, the metocestode stage of *T. ovis* was at slaughter observed in heart muscles, diaphragm and skeletal muscles from approx. a third of all sheep from one specific farm localised in South Jutland. The diagnosis was confirmed by molecular typing of the mitochondrial cytochrome c oxidase I (cox1) gene. Three newly imported dogs were suspected but the definitive host was unidentifiable. The finding is not regulated in the meat control procedures. However, infected meat is usually condemned due to aesthetic reasons causing economic losses. Thus, finding of *T. ovis* is of concern to sheep meat producers in the area, as the infection could have spread further on to other farms.


Keywords: Taenia ovis; Sheep measles; sheep; meat hygiene, intermediate host; Dogs; Cox1; Denmark

## 1. Introduction

*Taenia ovis* (also known as *Cysticercus ovis* or 'sheep measles') is a parasitic tapeworm belonging to the family Taeniidae. The adult stage resides in the small intestines of infected canids, including domestic dogs (Ransom, 1913) and red foxes (*Vulpes vulpes*) (Jenkins et al., 2014). The adult worms pass eggs onto pastures via faeces. Infestation in sheep (intermediate host) occurs when they inadvertently ingest the infective eggs when grazing on contaminated pasture. In intermediate hosts, the larval stage (also called metacestode stage or cysticerci) burrows through the intestinal mucosa and migrate via the bloodstream to the muscles. The predilection sites are cardiac muscles, diaphragm and masseter muscles. In heavy

infestations the larvae can also be observed throughout the skeletal musculature (Arundel, 1972). The infective cysticerci appear as small (approximately 4–6 mm) clear, cyst-like lesions in the muscles, each containing a single scolex (Arundel, 1972). Cysts are developed approx. two months following egg ingestion (Ransom, 1913), hereafter the cysts remain infective to canids for 1–2 months (Ransom, 1913). If raw cyst-holding meat is eaten by a canid, the adult parasite develops in the small intestine and the lifecycle continues. If the sheep is not slaughtered, the cysts calcify and form a small, innocuous nodule with a 'gritty' texture (DeWolf et al., 2014). This stage is commonly known as sheep measles due to the "spotty" appearance of meat containing the pale cystic lesions.

*Taenia ovis* infestations in sheep are of no apparent veterinary importance and in contrast to the other taeniid cestode, i.e. *Echinococcus multilocularis* and *E. granulosus*, canids infected with adult *T. ovis* pose no risk to human health. The importance of *T. ovis* is mainly of relevance to the meat industry is infestation in sheep with the intermediate stages of these cestodes leading to important financial losses, due to downgrading and condemnation of meat and hearts. *Taenia ovis* infestation is also a potential impediment to international trade of sheep meat (Jenkins et al., 2014). Both viable and calcified cysticerci are visible in the meat of infected animals, rendering it unacceptable for human consumption. The FAO guidelines recommend condemning of carcasses if lesions of *T. ovis* infestation are found in two of the usual inspection sites (masseter muscle, tongue, oesophagus, heart, diaphragm or exposed musculature).

*Taenia ovis* infestations appear worldwide, including Australia (Jenkins et al., 2014), Canada (DeWolf et al., 2014), China (Shi et al., 2016), England (Eichenberger et al., 2011), Ethiopia (Sissay et al., 2008), Iran (Hashemnia et al., 2016), Italy (Gori et al., 2015) and New Zealand (Lawson, 1994). Here we describe infestation with cysticerci of *T. ovis* in muscles of Danish sheep (*Ovis aries*). To our knowledge, this is the first publications describing this parasite infestation in Danish sheep.

# 2. Material and method

## 2.1 Case report

In April 2016, a veterinarian observed approx. 5 mm whitish cysts-like nodules in the cardiac- and skeletal muscles of sheep and lambs during meat inspection at a small abattoir in the South Jutland, Denmark. According to the veterinarian, the cysts appeared in one-third of the animals from one particular farm during spring 2016. During summer, the number of cyst-positive animals decreased, and in winter 2017, none of the lambs born in the summer 2016 had cyst-like nodules, while some of the ewes had calcified nodules.

In May 2016, the National Veterinary institute, Technical University of Denmark (DTU-VET) received three hearts with the aforesaid cyst-like nodules from the abattoir. The cardiac muscles were examined grossly as samples were taken. For histology, samples of myocardium with cysts were fixed in 10% neutral buffered formalin. After fixation the samples were prepared by routine methods, embedded in paraffin wax and sectioned at 3–5 µm. The sections were stained with haematoxylin and eosin for histopathological evaluation using a Leica DMRB microscope equipped with a MC120 HD camera and Leica Application Suite version 4.7.0, Leica Microsystems, Switzerland.

Cysts were dissected from the cardiac muscles and DNA of the cyst was extracted using QIAmp DNA mini kit according to the manufacturer's instructions (QIAmp DNA mini kit®, Qiagen, Hilden, Germany). The mitochondrial cytochrome c oxidase subunit 1 gene (cox1) was amplified using general primers according to Bowles et al. (1992). PCR amplicons were sequenced in both directions using ABI Prism Big Dye Terminator v 3.1 Sequencing Kit (Applied Biosystems, Foster City, CA), and the sequence was analysed according to the description of the manufacturer of Genetic Analyzer 3130 (Applied Biosystems, Appiera, Denmark). The consensus cox-1 sequence determined here was subjected to BLASTn analysis. Related sequences were retrieved from Genbank™ and alignment for further sequence analysis. Sequences were compared with other cox1 sequences of *Taenia* spp. present in Genbank™ to evaluate sequence similarity,

expressed as percentage of nucleotide identity. Phylogenetic relationships were inferred as described in Al-Sabi et al. (2013). Evolutionary relationships were inferred after constructing neighbourjoining (NJ) trees (Saitou and Nei, 1987), using the software MEGA6 (Tamura et al., 2013). Nucleotide sequence data reported in this paper are available in the GenBank™ under the accession number MG59480.

### 2.2 The sheep farm

The sheep and lambs originated from a farm in Skærbæk, in the South Jutland, Denmark, approx. 40 km north of the German border. The affected farm consists of approx. 400 ewes, raising 500 lambs per year for meat producing. Among others, the meat was used to produce Biologically Appropriate Raw Foods (BARF) for pets. The farm is located in an area where recently migrated wolves are living. Wolves have reappeared in Denmark within the last few years after around 200 years of absence. To protect the sheep from wolves, the sheep farmer imported three Maremmano Abruzzese puppies from Italy and Polen in June – December 2014, respectively. The farmer housed two other breeds: border collies and a labrador retriever. Both have access to the pastures where sheep are grazing, while the Maremmano Abruzzese lives together with the sheep. The dogs have been fed raw sheep meat originating from the farm. As the farm is located in known *E. multilocularis* risk area, all farm dogs are given anthelminthic treatment every second month. The Maremmano Abruzzese dogs were not dewormed prior to or upon arrival to Denmark, but entered straight into the treatment program at the farm.

Upon observation of cysts in the sheep, faeces from the three Maremmano Abruzzese dogs were analysed using a modified McMaster technique (Roepstorff and Nansen, 1998) at DTU-VET to reveal the presence of *Taenia* eggs, the analysis were however, after the dogs was dewormed.

## 3. Results and Discussion

The three hearts from sheep forwarded to DTU-VET contained 1–5 white cyst-like nodules of approx. 5 mm in diameter (Fig. 1). As the cysts only contained a pastious white material and/or were calcified, diagnosing

the parasite based on metacestode morphology was not possible. Histopathologically, the examined nodules were organized as abscesses containing eosinophilic masses of detritus and mineralized granules without clear signs of neither encapsulation or presence of vital parasites, as shown in Fig. 2a. However, a few eosinophilic structures resembling cross sections of necrotic helminths/cysticerci were observed (Fig 2b). The wall of the abscesses was relatively thin consisted of layers of mononuclear cells and fibrocytes. No inflammatory reaction was observed in the adjacent myocardium.

Based on the molecular analysis, the isolated cysts were identified as the cysticerci of *T. ovis*. This is the first published report of this parasite in Denmark, and no documentation on its previous presence is, to our knowledge, available.

Initially, the imported Maremmano Abruzzese dogs were considered the possible source, since the dogs were recently introduced on the farm and might have hosted the parasite prior to entering Denmark. The dogs had access to the sheep pasture and the possibility to contaminate the pastures with eggs. However, all farm dogs tested negative for taeniid eggs, but the dogs were treated with anthelminthic prior to examination. Thus, the pasture contamination could have occurred prior to treatment and testing.

Difference in nucleotide composition (390 bp) between Danish isolates and other isolates of *T. ovis* ranged 0.0 to 0.08%. Based in Phylogenetic analysis, the Danish isolates were aggregated in one branch together with isolates from Asia.

In this report, *T. ovis* infestation appeared as an isolated incident, which seemingly arose from import of infected dogs. Moreover, the lambs born in 2016 were free of cysts, indicating that the contamination is no longer present at the pastures. However, based on experimental studies, *T. ovis* eggs can remain viable on pasture for at least 300 days (Arundel, 1979). Single adult worm may produce 70,000-250,000 eggs per day (DeWolf et al., 2014), posing a risk for pasture contamination. However, pasture rotation might prevent re-infection on farms.

This current finding of *T. ovis* is of particular concern for this farm, as the meat is sold for raw consumption as lamb tatar to humans and BARF food for pets. Infested meat is usually considered inappropriate for human consumption (Rehbein et al., 2000), although the ovine muscular cystic lesions caused by *T. ovis* do not cause infection in humans, but are aesthetically undesirable in meat destined for human consumption.

Currently, there are no practical options for the treatment of the intermediate host. However, control measures include regular anthelmintic treatments of the farm dogs and omit feeding dogs with raw contaminated muscles or organs. Though, freezing the sheep meat and hearts for 10 days will inactivate *T. ovis* cystercerci rendering the meat safe to feed to dogs.

Although domestic dogs are considered the main definitive host for *T. ovis*, other canids has been proposed as potential definitive hosts, including wolves (Moks et al., 2006) and red foxes (Jenkins et al., 2014). In these publications, cestode identification was based on morphology, a method unusable when distinguishing between *T. krabbei* and *T. ovis*. The wolf is well-documented as final host for *T. krabbei* (Bryan et al., 2012; Craig and Craig, 2005; Holmes and Podesta, 1968). Since *T. krabbei* and *T. ovis* are morphological undifferentiable, morphological diagnosis of these parasites may be incorrect. However, in recent studies from Australia and Italy, molecular analysis have documented red foxes (*Vulpes vulpes*) (Jenkins et al., 2014) and wolves as sylvatic definitive host of *T. ovis* (Gori et al., 2015). Therefore, Danish wildlife could have been the source of the *T. ovis* infestation. However, the spread of *T. ovis* to wildlife like red foxes are less likely, since raw meat from production animals are not available to wildlife due to current legislation. In conclusion, although the definitive host was unidentifiable, is was suspected that to be three newly imported dogs, although there is a possibility that wild canid species, which has recently invaded Denmark was the source.

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## Figure captions

**Figure 1:** *Taenia ovis* cysticerci in the cardiac mucle from sheep (sheep measles).

**Figure 2:** Heart muscle from a slaughtered sheep with a subpicardial cyst-like nodule. The nodule is organized as an abscess containing eosinophilic masses of detritus and mineralized granules (arrow). a) The wall of the abscess (arrow head) is relatively thin consisting of a few layers of mononuclear cells and fibrocytes. No inflammatory reaction was observed in the adjacent myocardium (star). H&E, bar 1mm. An eosinophilic structure similar to a cross section of necrotic helminth/cysticerci with a homogeneous cuticle (arrow head) is seen in the centre of the abscess (square). b) H&E, bar 200µm.

**Figure 3:** Phylogenetic tree inferring the evolutionary relationship between a Danish isolate of Taenia ovis with other T. ovis isolates worldwide, and two closely related Taenia species as out-groups. The tree was inferred using the Neighborjoining method, based on analysis of 390 bp of the mitochondrial cox-1 gene. The scale bar indicates distance.

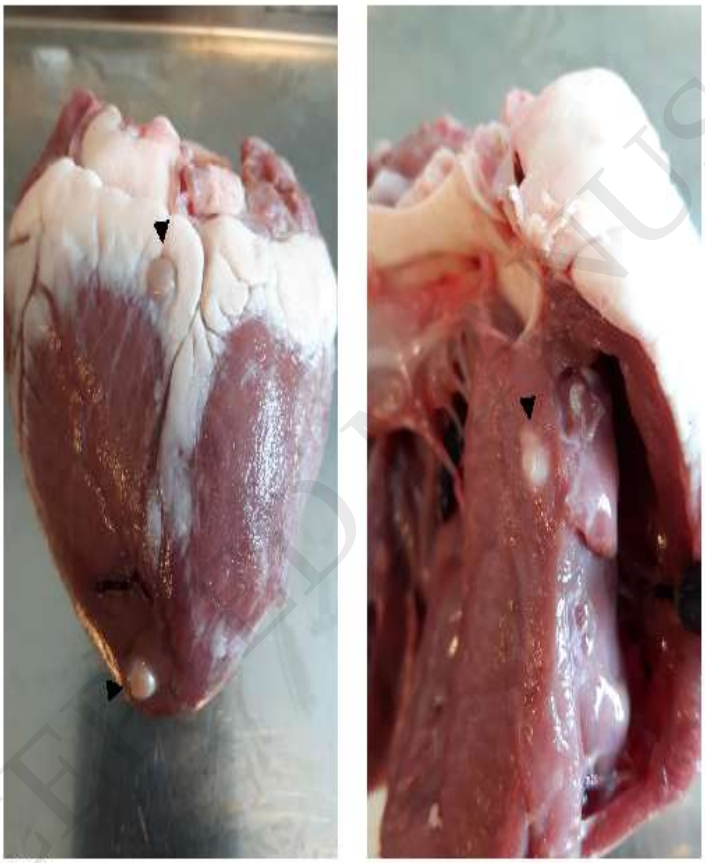

Figure 1

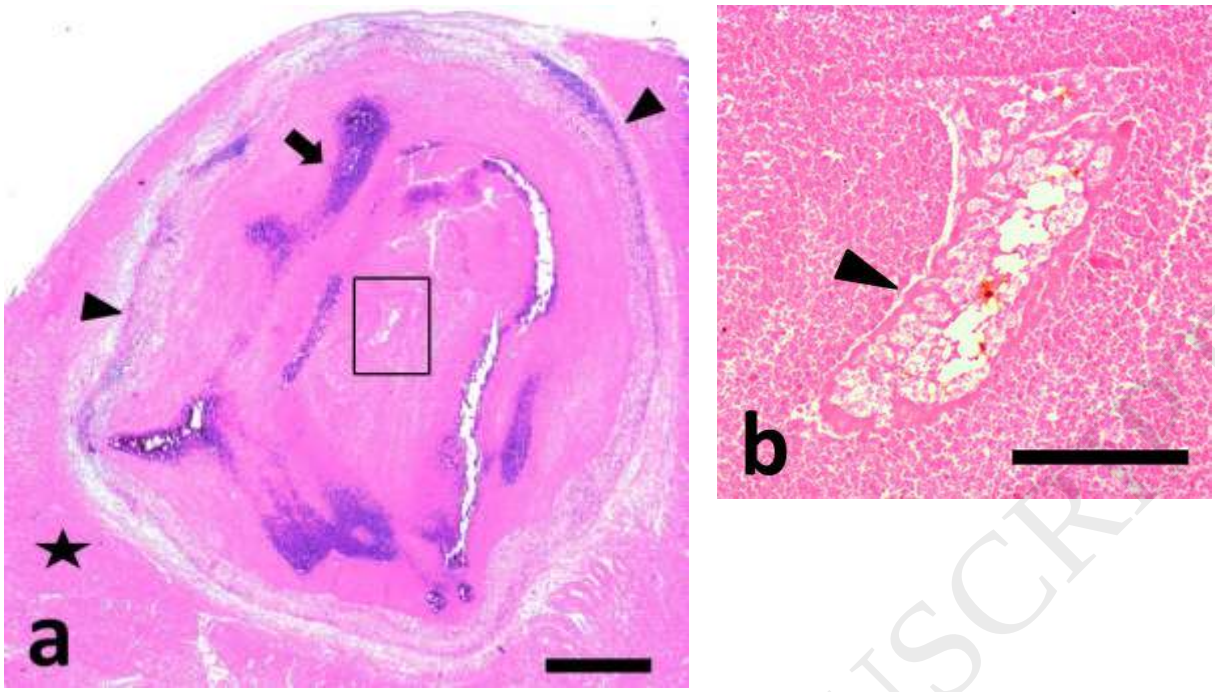


**Figure 2:**

Figure 3