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Reappearance of *Taenia ovis krabbei* muscle cysts in a roe deer (*Capreolus capreolus*) in Denmark after 60+ years

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Abstract

The present report describes the reappearance of *Taenia ovis krabbei* in a roe deer from Denmark after more than 60 years. The cysticerci were isolated from the thigh muscle of the deer, and the diagnosis was based on histostological analysis, morphology of the rostellar-hooks as well as molecular typing of the mitochondrial cytochrome c oxidase I (*cox1*) gene. The exact definitive host was not revealed in this report, but domestic dogs may play a role of the definitive host in the area. This finding is of concern to hunters and deer meat producers, since the infected meat is usually condemned due to aesthetic reasons.
Keywords: *Taenia ovis krabbei*, deer, intermediate host, rostellar hooks, *cox1*, meat hygiene, Denmark

A sequence of the isolated *Taenia ovis krabbei* has been uploaded in GenBank with accession number: JX560319.

**Introduction**

*Taenia ovis krabbei* is a parasite with a sylvatic life cycle, in which carnivores are the definitive hosts and Cervids are the intermediate hosts (Jones and Pybus, 2001). *Taenia ovis krabbei* is morphologically very similar to its kin subspecies; *Taenia ovis* (Priemer et al., 2002) which were separated by Verster, (1969) since the later subspecies infects farmed small ruminants but not Cervids (Sweatman and Henshall, 1962). Accidental foraging on pasture contaminated with eggs of *T. o. krabbei* is the primary cause of infection in the intermediate hosts. The larval stage of this parasite (synonymous with *Cysticercus tarandi*) usually develops in heart and skeletal muscles and may cause pathological changes and severe illness in infected deer (Christensen and Roth, 1949). The life cycle is completed when the definitive hosts ingest infected meat of the intermediate hosts (Sweatman and Henshall, 1962). The consumption of game meat infected with *T. o. krabbei* is not considered a potential zoonosis (Jones and Pybus, 2001; Hoberg, 2002). However, for aesthetic
reasons the infected meat is not regarded of high quality and may be discarded upon meat inspection in abattoirs (Rehbein et al., 2000).

In the current report we describe an infection with cysticerci of *T. o. krabbei* in the thigh muscle of a roe deer (*Capreolus capreolus*).

**Case description**

**Origin of the roe deer**

In May 2012, a male roe deer was killed by a hunter in Pajheden forest (57°17'33.24''N; 10°12'47.15''W). This small forest is located near the small village of Brønden in the northern part of Jutland, Denmark. The population of roe deer in Pajheden forest is unknown, but according to the owner several bucks are regularly observed, in addition to a fox couple, few hares, lots of wood pigeons, a few partridges, pheasants and a pair of trailing owls. The forest is open to the public, and leached dogs are allowed as well as hunting dogs during deer hunts.

**Parasitological examination**

Following observation of white spots in the muscles of the roe deer, a sample of the thigh muscle was submitted to the National Veterinary Institute, Technical University of Denmark, for further identification. The muscle sample was analyzed macroscopically by visual examination, and subsequently subsections were formalin-fixed, paraffin-embedded and subjected to
histopathological evaluation. The morphology of rostellar hooks isolated from one cyst was examined under light microscopy and the diagnosis was done according to Loos-Frank (2000).

**Molecular analysis**

The DNA of the morphologically examined cyst was extracted using a commercial kit according to the manufacturer’s instructions (QIAmp DNA mini kit®, Qiagen, Hilden, Germany), and a partial sequence of the mitochondrial cytochrome c oxidase subunit 1 gene (coxl) was amplified using general primers according to Bowles et al. (1992), including DNA of a Danish isolate of a trematode (*Alaria alata*) as positive control and Milli-q water containing mastermix as negative control. 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 analyzed 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 (http://blast.ncbi.nlm.nih.gov) to establish the 'top hits' to all nucleotide sequences available in current databases and sequence identities (in %) was calculated by pairwise comparisons.

Subsequently, the consensus pcox-1 sequence was aligned (over 382 bp length) with the selected subset of closely related sequences, including *Taenia ovis krabbei, Taenia ovis, Taenia arctos* and *Taenia saginata* (outgroup) (with the following GenBank acc. nrs. respectively, EU544573, EU544575, JF261321, JF261327, JF261322, JX134122, GU252131, GU252130 and AB645845).
Phylogenetic relationships were inferred based on analyses employing Bayesian inference (BI), Maximum Parsimony (MP) and the Neighbor-Joining (NJ) methods. Bayesian inference was calculated using the Monte Carlo Markov Chain (MCMC) method of MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). The likelihood parameters were set based on the Akaike Information Criteria (AIC) test in Modeltest v3.7 (Posada and Crandall, 1998). The general time-reversible model of evolution, with gamma-distribution and a proportion of invariable sites (GTR + Γ + I), was utilized for the analysis of the cox-1 sequence data. The estimates of the base frequencies, the substitution rate model matrix and the proportion of invariable sites were fixed. Posterior probabilities (pp) were calculated for 2,000,000 generations, utilizing four simultaneous tree-building chains, with every 100th tree being saved. At this point, the potential scale reduction factor (PSRF) approached one.

The MP method was obtained using the Close-Neighbor-Interchange algorithm with search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates) (Nei and Kumar, 2000). The NJ method, on the other hand, was obtained according to Saitou and Nei, (1987). For both MP and NJ trees, the percentage of replicate tree in which the associated taxa clustered together in the bootstrap test (10,000 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and were in the units of the number of base substitutions per site. The trees were drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. Evolutionary analyses for MP and NJ were conducted using the software MEGA5 (Tamura et al., 2011).
Results and Discussion

Numerous oval, white cysts of approximately two to four mm in diameter were observed by macroscopic analysis (Figure 1, A & B). Histopathological examination of embedded muscle samples showed a cysticercus with a bladder membrane and watery, transparent fluid (Figure 1, C). The encapsulation of the cysticercus varied from a thin, non-cellular collagen layer to a granulomatous inflammation with large, foreign body giant cells. Apart from that no inflammatory reaction was observed in the muscles, but a low number of sarcocysts were noticed. The characteristic shape and size of the recovered rostellar hooks (Figure 1, D) showed typical morphology of either *T. o. krabbei* or *T. o. ovis*, but not of any other *Taenia* spp.; large hooks \((N = 17)\) had a blade length = 69 µm and a handle length = 54 µm (Figure 1, E), whereas small hooks \((N = 17)\) had a blade length = 60 µm and a handle length = 13 µm (Figure 1, F).

Phylogenetic trees constructed by the various methods used in the current study were very similar in topology and therefore presented in one tree showing the nodal support for each of the methods (Figure 2). Accordingly, the Danish isolate described here clearly grouped together with other isolates of *T. o. krabbei* in one node that had a relatively high posterior probability value for BI (0.88), and high bootstrap values for NJ and MP (0.99 and 1.00, respectively), reflecting high reliability of the data. This node was clearly distant from the morphologically similar *T. ovis* and the recently described *T. arctos* (Haukisalmi et al., 2011), which were grouped together in one branch with relatively higher posterior probability value for BI (0.98) and high bootstrap values for NJ and MP (0.98 and 0.93, respectively). The Danish isolate reported here was closely related to *T. o. krabbei* isolated from Gray wolves from Finland (haplotypes K2 and K1; Lavikainen et al., 2011).
and Sweden (haplotype K8), and slightly more distantly related to *T. o. krabbei* isolated from Arctic foxes from Norway (haplotype K10). The relatively lower posterior probability value for BI (0.8) and bootstrap values for NJ and MP (0.73 and 0.67, respectively) of the branch containing *T. o. krabbei* isolated from Arctic foxes may be related to the remote geographic origin of these isolates compared to those from other areas of the Nordic peninsula, or could be related to the host origins of the isolates. Unfortunately, *cox1* sequences of German isolates of *T. o. krabbei* were not available in GenBank when we performed this analysis.

Based on the previous morphologic and molecular analysis, the isolated cysts were identified as *T. o. krabbei*. This is the first report of this parasite in the area of Jutland, Denmark, and a reappearance of the parasite in roe deer in Denmark after more than 60 years (Christensen and Roth, 1949). This current finding of *T. o. krabbei* is of particular interest to game meat retailers since infected meat is usually not considered fit for human consumption (Rehbein et al., 2000).

In Europe, infections with cysticerci of *T. o. krabbei* were frequently reported in red deer, roe deer, fallow deer, mouflon, reindeer, caribou and muskox (Kolar et al., 1978; Murai and Sugar, 1979; Clausen et al., 1980; Bye, 1985; Rehbein et al., 2000; Rehbein et al., 2001; Rehbein et al., 2002; Shimalov and Shimalov, 2003; Cuyler et al., 2005; Raundrup et al., 2012). In Argentina, the introduction of infected red deer was suggested as a potential route for spreading of the infection into non-endemic areas (Flueck et al., 2006). Cysticerci of *T. o. krabbei* were previously isolated from roe deer in most of the federal states in Germany including the state that shares the border with Denmark; however, prevalence rates are unknown (Rehbein et al., 2000). We do not think that infected farmed small ruminants are a likely route of transmission since attempt to establish
infection with *T. o. krabbei* in sheep and goats were unsuccessful (Sweatman and Henshall, 1962; Murai and Sugar, 1979). Other routes of transmission e.g., via deer transport are seldom practised in the study area (Mariann Chriél, personal communication). Therefore, we suggest that introduction of *T. o. krabbei* to the area via a definitive host is the most probable source of infection in the present case as this region is more than 300 km from the German border. Nevertheless it is also possible that deer infections with *T. o. krabbei* are present but unnoticed in the whole area of Jutland.

Arctic foxes (Bye, 1985; Kapel and Nansen, 1996; Stien et al., 2010) and wolves (Rausch et al., 1983) are generally acknowledged as the main definitive hosts of *T. o. krabbei* in the arctic tundra. In mainland Europe, *T. o. krabbei* is primarily a parasite of wolves (Shimalov and Shimalov, 2000; Priemer et al., 2002, Moks et al., 2006; Bagrade et al., 2009). Since these two carnivores have not been present in Denmark for several decades, other definitive hosts might be the source of infection.

*Taenia ovis krabbei* is seldom reported among red foxes in Europe, despite its co-existence with wolves in many countries. In the literature, adult worms of *T. o. krabbei* (identified as *T. cervi*) were isolated from a red fox from Ukraine (Kornyushin et al., 2011) and a red fox in Germany (Lucius et al., 1988). The latter case may have to be revisited since the described morphologies of the mature and gravid segments are indistinguishable from other *Taenia* spp. (Cram, 1926; Verster, 1969; Loos-Frank, 2000). With the exception of *T. o. ovis*, differentiation of *T. o. krabbei* from other *Taenia* spp., can be done based on the morphology of rostellar hooks (Priemer et al., 2002), otherwise the use of molecular methods is inevitable (Lavikainen et al., 2010; Kutz et al., 2012).
Nonetheless, the successful establishment of *T. o. krabbei* in an experimentally infected fox (Murai and Sugar, 1979) may suggest the potential role of the red fox as a definitive host in Europe.

In the current case, domestic dogs in the hunting area may have been the definitive hosts. The dog is known to be a successful definitive host of *T. o. krabbei* based on observations from natural (Cram, 1926; Christensen and Roth, 1949; Sweatman and Henshall, 1962; Verster, 1969; Murai and Sugar, 1979) and experimental infections (Moniez, 1879; Shaw, 1947; Christensen and Roth, 1949). In Denmark, dogs infected with *T. o. krabbei* were previously reported from the islands of Zealand and Lolland (Christensen and Roth, 1949). In addition, international pet travel without proper control of animals tends to be an increasing problem and a risk for transmission of pathogens across borders (Defra, 2010; Davidson and Robertson, 2012). Thus, we expect a potential role for domestic dogs in the spread of infectious taeniid eggs including eggs of *T. o. krabbei* in the environment.

The current reappearance of *T. o. krabbei* in a roe deer from a remote area in Denmark emphasizes the importance of this infection for hunters and producers of deer meat, and suggests the possibility of an under estimated prevalence of this parasite among deer as well as carnivores.

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Conflict of interest

All authors declare no financial or personal conflicts of interest.
References:


Figure captions:

Figure 1: Cysticerci of *Taenia ovis krabbei* in a roe deer muscle as they appear by gross morphology, histology and microscopy. (A): External view of the thigh muscle showing the presence of three cysticerci. (B): Section through the thigh muscle showing a cysticercus. (C): Histological section of a cysticercus stained with hematoxylin & eosin staining, scale bar = 0.5mm. (D): Microscopy of the rostellum showing large (E) and small hooks (F).

Figure 2: Phylogenetic relationship of *Taenia ovis krabbei* based on *cox-1* sequence data determined herein *, together with selected reference sequences for *Taenia ovis krabbei* and the related taeniid cestodes, presented with its scientific name, (host), country of origin then GenBank acc. nr. Relationships were inferred based on analyses employing Bayesian Inference (BI), distance-based Neighbour Joining (NJ) and Maximum Parsimony methods, with given nodal supports of posterior probability for BI (first value) and a bootstrap value for NJ (second) and MP (third). Haplotype designations according to Lavikainen et al., (2011) are shown to the right of taxons. The scale bar indicates distance.
Taenia saginata (Human) Thailand AB645845

Taenia ovis (Sheep) Iran JX134122

0.98/0.98/0.93

Taenia arctos (Eurasian elk) Finland GU252130

Taenia arctos (Eurasian elk) Finland GU252131

0.88/0.99/1.00

Taenia krabbei (Gray wolf) Finland JF261322 K1

Taenia krabbei (Gray wolf) Sweden JF261327 K8

Taenia krabbei (Gray wolf) Finland JF261321 K2

Taenia krabbei (Roe deer) Denmark JX560319 *

Taenia krabbei (Arctic fox) Norway EU544575 K10

0.80/0.73/0.67

Taenia krabbei (Arctic fox) Norway EU544573 K10

0.03