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First report of pulmonary cysticercosis caused by *Taenia crassiceps* in a Cape fur seal (*Arctocephalus pusillus*)

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ABSTRACT

The cestode *Taenia crassiceps* parasitizes in the intestine of domestic and wild carnivores, especially in red foxes. Usually, the metacestode stage, also known as Cysticercus longicollis, is located in muscles, peritoneal and pleural cavity of wild rodents. In this case, larval stages were found in a female Cape fur seal, which lived in a German zoo since June 1998. In January 2019, the animal presented clinical signs in terms of inappetence and reduced mobility and, within a short time, it developed dyspnoea and died. Pathological and parasitological examinations were performed. In a large mass of the right thoracic wall and in nodular lung lesions, metacestodes with numerous protoscoleces were identified. Morphological and molecular analyses led to the diagnosis of a *Taenia crassiceps* infection. Probably, the urban fox population was the source of infection. Thus, regarding the zoonotic potential of this cestode, a regularly performed parasitological examination of pet dogs is recommended.

1. Introduction

Taenia crassiceps is a cestode species that inhabits the small intestine of domestic and wild carnivores in the Northern hemisphere. The red fox (*Vulpes vulpes*) is considered as the most common definitive host (Alić et al., 2017). In its metacestode stage (Cysticercus longicollis), the cestode parasitizes in the muscles, peritoneal and pleural cavity of numerous mammal species, but wild rodents, especially the common vole (*Microtus arvalis*), are mentioned as natural intermediate hosts in Europe (Konjević et al., 2016). A special feature of *T. crassiceps* is its ability to reproduce asexually by budding at the larval stage and thus, the parasite is able to maintain and colonize its host for long periods of time (Peón et al., 2013). Cases of *T. crassiceps* cysticercosis are also described in lemurs (Dyer and Greve, 1998; Luzón et al., 2010; Alić et al., 2017), a Cape ground squirrel, a Senegal bushbaby (Hofmannova et al., 2018), a Nilgiri langur (Bleyer et al., 2018) and a chinchilla (Basso et al., 2014). Even though seldom, carnivores may also serve as intermediate hosts and cases are described in dogs, cats and foxes (Konjević et al., 2016; Whipp et al., 2017). Humans rarely serve as intermediate host, however, an increasing number of zoonotic infections have emerged in recent years, whereby infection is thought to occur after consumption of food or water contaminated with infective ova shed in carnivore faeces (Ntoukas et al., 2013). To our knowledge there is no case of Cysticercus longicollis described in marine mammals before, so this is the first report of *Taenia crassiceps* in a Cape fur seal (*Arctocephalus pusillus*).

2. Materials and methods

2.1. Clinical presentation

The herein presented female Cape fur seal was caught at the South African coast and brought to the Zoo Leipzig, Saxony, Germany, in June 1998. The fur seal gave birth to 6 pups, the last one was born in 2014. In November 2018, a swelling in the right shoulder region of the fur seal was recognized, but the animal showed no impairment of general condition or mobility. Therefore, no further diagnostics or treatment was performed. In the end of January 2019 the animal presented clinical signs in terms of inappetence, reduced mobility and unusually long stays ashore. A treatment trial with butorphanol failed to effect an improvement of the clinical presentation. Furthermore, the fur seal developed dyspnoea and died within a short time.

2.2. Pathological examination

Necropsy was performed immediately after death. Specimens of the

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gut and the altered tissue of the lung and the thoracic wall were collected for parasitological examinations. Representative tissue samples were formalin fixed, embedded in paraplast, sectioned at 3–4 µm and stained with hemalum and eosin (Mulisch and Welsch, 2010). The slides were microscopically screened for morphological alterations.

2.3. Parasitological examination

The content of the intestines was examined by flotation/sedimentation technique. Additionally, the intestines were probed macroscopically. Metacestodes were isolated from the lung and the thoracic wall mass and morphologically analysed by microscopy. Furthermore, DNA was isolated from the metacestodes of both tissues using the QIAmp DNA Mini Kit® (QUIAGEN, Hilden, Germany) following the manufacturer's instructions. For sequence analysis, PCR was performed using primers published by Bowles et al. (1992) for a fragment of the cytochrome oxidase 1 gene (COX 1): forward 5′TTTTTTGGGCATCCT GAGGTTTAT3′ and reverse 5′TAAAGAAAGAACATAATGAAAATG3′ with the following conditions: 0.1 µM of each primer, 0.1 U of DreamTaq DNA polymerase (Thermo Scientific, Waltham, USA) and the manufacturer's recommended reaction buffer. Thermal protocol was performed as follows: 95 °C for 15 min; 95 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s (35 x) followed by 72 °C for 5 min. After running gel-electrophoresis, the gel was stained with ethidium bromide and bands were visualised by UV light. Purification of PCR products was done by using the GeneJET PCR Purification Kit (Thermo Scientific). Sequencing was done by a commercial company (Microsynth Seqlab, Göttingen, Germany). The obtained sequences were visually observed with MEGA version X (Kumar et al., 2018) and compared with those available in the GenBank database by BLASTn analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

3. Results and discussion

On gross examination, the right thoracic wall showed a large fluctuating mass (approx. 40 × 30 × 20 cm) reaching from the subcutis to the thoracic cavity (Fig. 1a). Incision revealed large amounts of beige-whitish to transparent material. The lung had numerous beige-whitish nodules measuring up to 3 cm in diameter containing the same material as described above. Several regional lymph nodes of the lung appeared moderately to markedly enlarged. The gastrointestinal tract contained small to moderate amounts of ingesta without signs of inflammatory and infectious processes, respectively. Furthermore, no parasite stages were detectable in the intestines. Histologically, the large mass of the thoracic wall as well as the nodular lung lesions consisted of

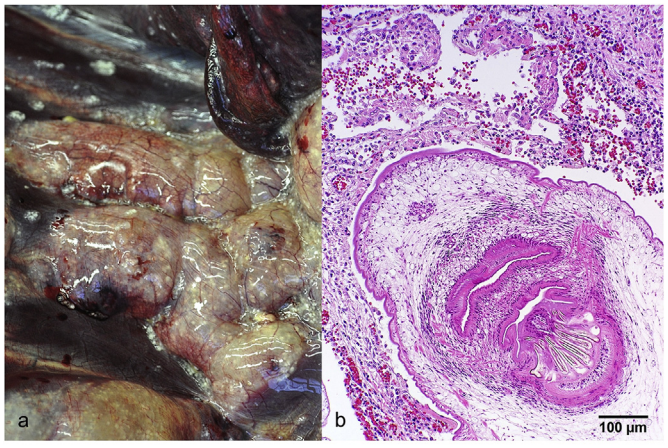

Fig. 1. a) Large mass in the right thoracic wall. b) Protoscolex in the lung showing rostellar hooks. A mild chronic inflammation is obvious in the surrounding lung tissue.

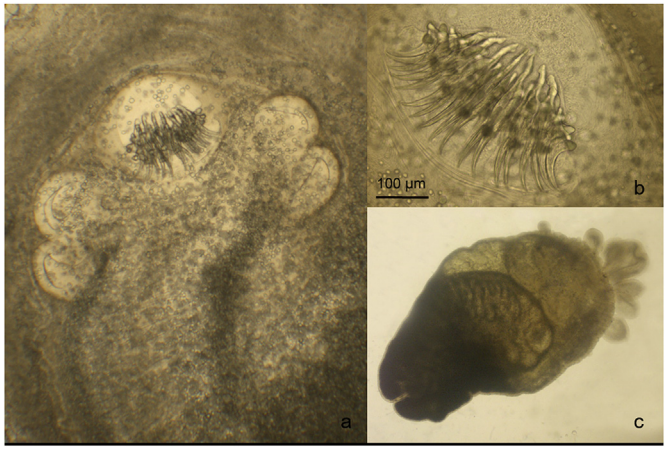

Fig. 2. a) Cysticercus longicollis scolex with rostellar hooks and four suckers. b) Apical rostellum with two circular rows. c) Exogenous budding of Cysticercus longicollis in the subcutis of a Diana monkey.

metacestodes with numerous protoscoleces (Fig. 1b). In the surrounding tissue, a mild to moderate chronic inflammation could be detected. Morphological examinations of the cysticerci from the lung and the thoracic wall mass led to the diagnosis of a *Taenia crassiceps* infection due to shape and size of the rostellar hooks, which were found as a part of the protoscoleces, as well as four suckers (Fig. 2a). The size of the small hooks was 130 µm, while the large hooks had a size of 160 µm (Fig. 2 b). Konjević et al. (2016) summarized known literature describing the size of the large hooks from various hosts in a range of 129–210 µm, while the small hooks range from 95 to 157 µm. The molecular analysis confirmed the morphological examinations. The herein obtained partial sequence of the COX 1 gene (366 bp) (accession no: **MK757442**) from the lung tissue fit in with *T. crassiceps* sequences from the GenBank (NCBI BLASTN tool). A homology of 100% was obtained, when an exemplary *T. crassiceps* COX 1 gene sequence (accession no. KY321321.1) was used for alignment (Fig. 3). Although the larval stage of *T. crassiceps* is predominantly found in common voles (Konjević et al., 2016), cases of Cysticercus longicollis were also described in unusual hosts like black or ring-tailed lemurs (Dyer and Greve, 1998; Alić et al., 2017). Furthermore, cysticercosis was observed in a Cape ground squirrel and a Senegal bushbaby, both kept in the same zoo in the Czech Republic (Hofmannova et al., 2018). In 2018, another case of *T. crassiceps* infection was diagnosed in the Zoo Leipzig (unpublished), and cysticerci were found subcutaneously in a Diana monkey (*Cercopithecus diana diana*) and examined morphologically (Fig. 2c). Considering the presence of protoscoleces, in the herein presented case, the examined cysticerci were probably infective for a definitive host. The finding of fertile metacestodes is in accordance with former reports of Cysticercus longicollis in other animal species and humans (Dyer and Greve, 1998; Bröjer et al., 2002; Wünschmann et al., 2003; Aghamohammadi et al., 2008; Ntoukas et al., 2013). The number of *Taenia crassiceps* cysticercosis in animals and humans in Europe is growing, probably as a result of environmental contamination by parasite eggs distributed through the dense population of (also urban) red foxes (Hofmannova et al., 2018). Foxes are present in the City of Leipzig and also in the Zoo Leipzig and thus, they could probably be the source of infection. The prevalence of *T. crassiceps* infection among foxes was reported to be relatively high in Germany (Ntoukas et al., 2013), varying between 24% in south-west Germany (Loos-Frank and Zeyhle, 1982), 28.5% in west Germany (Ballek et al., 1992), 15% in Berlin (Schöffel et al., 1991), and 17.7% in Saxony-Anhalt (east Germany) (Pfeiffer et al., 1997). However, the enclosure of the fur seals' prevents direct access for foxes. Thus, another way of transmission had to take place, so maybe the ova shed got into the furs seals' tank or into the enclosure by vectors. Due to its proliferative cysticercus, ingestion

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Query  1    ATGAATTGTGATTCTTTTGGTTTTTATGGATTGTTATTTGCTATGTTTTCAATAGTTTGT  60
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  79   ATGAATTGTGATTCTTTTGGTTTTTATGGATTGTTATTTGCTATGTTTTCAATAGTTTGT  138

Query  61   TTAGGTAGGAGTGTTTGGGGTCATCATATGTTTACGGTTGGTTTAGATGTTAAGACTGCT  120
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  139  TTAGGTAGGAGTGTTTGGGGTCATCATATGTTTACGGTTGGTTTAGATGTTAAGACTGCT  198

Query  121  Gtttttttt AGTTCTGTTACTATGATTATAGGAGTACCTACAGGTATAAAGGTGTTTACT  180
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  199  GTTTTTTTTAGTTCTGTTACTATGATTATAGGAGTACCTACAGGTATAAAGGTGTTTACT  258

Query  181  TGATTGTATATGCTTTTAAATTCGCGTGTGAACAAGAGTGATCCTATATTGTGGTGAATT  240
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  259  TGATTGTATATGCTTTTAAATTCGCGTGTGAACAAGAGTGATCCTATATTGTGGTGAATT  318

Query  241  GTTTCTTTTATAGTTTTATTTACGTTTGGTGGTGTTACTGGAATAGTATTGTCTGCTTGT  300
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  319  GTTTCTTTTATAGTTTTATTTACGTTTGGTGGTGTTACTGGAATAGTATTGTCTGCTTGT  378

Query  301  GTATTAGATAAAGTTCTTCATGATACTTGATTTGTTGTTGCTCATTTTCATTATGTTCTT  360
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  379  GTATTAGATAAAGTTCTTCATGATACTTGATTTGTTGTTGCTCATTTTCATTATGTTCTT  438

Query  361  TCTTTA  366
           ||||||
Sbjct  439  TCTTTA  444
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Fig. 3. Alignment result for the partial sequence of the COX 1 gene (fur seal) with an exemplary *T. crassiceps* COX 1 gene sequence (accession no. KY321321.1), obtained from NCBI BLASTN tool. Homology was 100% (query: KY321321.1, sbject: herein obtained sequence).

of only one or a few eggs can result in massive infections, so this parasite is not only a threat for animals, but also bears a zoonotic risk (Ballweber, 2009). Ntoukas et al. (2013) described that most of the infections in humans have been reported in southern Germany and France, but also in Switzerland, Austria and North America humans became infected. The authors concluded that similar to the distribution of alveolar echinococcosis in Europe, a contiguous area with micro foci of transmission could hypothetically be possible (Ntoukas et al., 2013). Most of the human cases took place in immunocompromised patients, in which *T. crassiceps* cysticerci showed a tropism for subcutaneous and muscular tissues (Flammer Anikpeh et al., 2014). All patients were dog owners (Flammer Anikpeh et al., 2014), and although the source of human infections is generally unknown, at least one case in North America has been linked to the family dog (Ballweber, 2009). Dyachenko et al. (2008)detected taeniid eggs in 0.3% of canine faecal samples from Germany. The authors demonstrated that the spectrum of *Taenia* spp. in dogs from Germany seems to reflect that of the fox population, including *T. crassiceps*, and suggested a similar behaviour regarding the consumption of small rodents as intermediate hosts (Dyachenko et al., 2008). Veterinarians should remember that domestic dogs are definitive hosts for multiple species of *Taenia*, including *T. crassiceps* (Ballweber, 2009), and therefore, to reduce the infection risk for humans, parasitological diagnostics should be performed regularly, especially in dogs with higher risk of infection like hunting dogs.

4. Conclusion

This is the first report of *Taenia crassiceps* cysticercosis in a Cape fur seal. Although the way of transmission remains unclear, the urban fox population, living next to the zoo, is probably related to this infection. In recent years an increasing number of cases of Cysticercus longicollis in animals and humans has been described. Thus, although these cases are still relatively rare, a regularly performed parasitological examination of pet dogs is recommended to protect them and their owners.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijppaw.2019.07.006.

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