Heartworms in *Halichoerus grypus*: first records of *Acanthocheilonema. spirocauda* (Onchocercidae; Filarioidea) in two grey seals from the North Sea

Kristina Lehnert¹, Insa Herzog¹, Joy Ometere Boyi¹, Stephanie Gross¹, Peter Wohlsein², Christa Ewers³, Ellen Prenger-Berninghoff³, Ursula Siebert¹

¹ Institute for Terrestrial and Aquatic Wildlife Research, University of Veterinary Medicine Hannover

² Department of Pathology, University of Veterinary Medicine, Hannover,

³ Institute of Hygiene and Infectious Diseases of Animals, Justus-Liebig-University Giessen, 35392 Giessen, Germany

**Corresponding author:** Kristina Lehnert, Kristina.Lehnert@tiho-hannover.de
Abstract

The assumed definitive host of the heartworm *Acanthocheilonema spirocauda* (Onchoceridae; Filarioidea) is the harbour seal (*Phoca vitulina*). This filaroid nematode parasitizing in cardiac ventricles and blood vessel lumina of harbour seals (*Phoca vitulina*) has a low prevalence and seldom causes severe health impacts. The seal louse (*Echinophthirius horridus*) is the assumed intermediate host for transmission of *A. spirocauda* filariae between seals, comprising a unique parasite assembly conveyed from the terrestrial ancestors of pinnipeds. Although grey seals (*Halichoerus grypus*) are infected by seal lice, heartworm infection was not verified. Analysing a longterm dataset compiled over decades (1996-2021) of health monitoring seals along the German coasts comprising post mortem investigations and archived parasites, two cases of *A. spirocauda* infected male grey seals were detected. Tentative morphological identification was confirmed with molecular tools by sequencing a section of mtDNA COI and comparing nucleotide data with available heartworm sequence. This is the first record of heartworm individuals collected from the heart of grey seals at necropsy. It remains puzzling why heartworm infection occur much less frequently in grey than in harbour seals, although both species use the same habitat, share mixed haul-outs and consume similar prey species. If transmission occurs directly via seal louse vectors on haul-outs, increasing seal populations in the North- and Baltic Sea could have density dependent effects on prevalence of heartworm and seal louse infections. It remains to be shown how species-specificity of filarial nematodes as well as immune system traits of grey seals influence infection patterns of *A. spirocauda*.

**Keywords:** harbour seal (*Phoca vitulina*), seal louse (*Echinophthirius horridus*), stranding network, health monitoring, molecular parasitology, insect vector
Introduction

Grey seals (*Halichoerus grypus*) and harbour seals (*Phoca vitulina*) are the two resident seal species in the German North- and Baltic Sea with numbers increasing over the last decades (Reijnders et al., 2005; Galatius et al., 2021). In the last century grey and harbour seal populations had been depleted by hunting, habitat loss and contaminant exposure (Wolff 2000; Lotze 2007; Silva et al., 2021) in both the North- and Baltic Sea. Additionally, North Sea harbour seals were reduced by phocine distemper epidemics (1988 and 2002) (Härkönen et al., 2006) and influenza A virus, serotype H7N10 (2014) (Bodewes et al., 2015). Seals are infected by a multitude of parasites consisting mostly of trophically transmitted endoparasitic helminths with multi-stage life cycles and varying prevalences, intensities, and pathological impacts. The heartworm *Acanthocheilonema (A.) spirocauda* is a filarial nematode (Onchocercidae; Filarioidea) that was first described from the heart of harbour seals (Leidy 1858; Anderson 1959) and later from the heart and surrounding blood vessels of multiple seal species including ringed (*Pusa hispida*), harp (*Phoca groenlandica*) and hooded seals (*Cristophora cristata*) (Measures et al., 1996) from Canada and North America (Dunn 1976). *A. spirocauda* is common in harbour seals along the coasts of The Netherlands (Van den Broek & Wensvoort 1959), Germany (Claussen et al., 1991; Lehnert et al., 2015), Denmark and Sweden (Lunneryd 1992); see also Leidenberger, S. et al. (2008) for a review. Recently, *A. spirocauda* was recorded from monk seal (*Monachus monachus*) in the Mediterranean (Papadopoulos et al., 2010). The seal louse *Echinophthirius (E.) horridus* (Anoplura; Insecta) has been hypothesized to be the intermediate host for heartworm filariae that are transmitted via the blood meal and undergo several moults in the insect vector (Geraci et al., 1981; Lehnert et al., 2015; Ebmer et al., 2022) before becoming infective and being directly transmitted to a potential new host via infected louse vectors - transferred e.g. during haul-out on sandbanks between seal individuals. *A. spirocauda* was reported from the right chamber and atrium in harbour seals from the
German Wadden Sea at 12% prevalence (Lehnert et al., 2015), while 25% (Borgsteede et al., 1991) and 32% prevalence (Claussen et al., 1991) were found after the 1988 PDV epidemic. Around the same time, 11% *A. spirocauda* prevalence was reported from the Kattegat/Skagerrak and Baltic Sea region (Lunneryd 1992). Heartworm usually does not cause severe health effects in harbour seals, although a perforated atrium and subsequent mortality was described in one case (Lehnert et al., 2015). So far it was assumed that grey seals do not get infected by *A. spirocauda* (Leidenberger et al., 2008) although one study suggested the potential presence of heartworm in grey seal hosts (Keroack et al., 2018). This study reports *A. spirocauda* for the first time in the heart of two grey seals found stranded on the German coastline and complemented morphological identification of the specimens with molecular techniques for unambiguous species identity.

**Materials and methods**

Within a coordinated stranding network established in 1990, post-mortem investigations on marine mammals found on the coasts of the German Federal State of Schleswig-Holstein (S-H) were performed at the Institute for Terrestrial and Aquatic Wildlife Research (ITAW) (Siebert et al., 2007). Dead stranded animals were retrieved and terminally sick animals were mercy-killed by licensed seal hunters for animal welfare reasons (Siebert et al., 2007). Decomposition status (DCC), with DCC1 being very fresh animals, DCC2 fresh, DCC3 moderate decomposition, DCC 4 advanced decomposition and DCC 5 mummified animals (Ijsseldijk et al. 2019) and nutritional status were assessed during necropsy which was performed in accordance to an established protocol (Ijsseldijk et al., 2019). Age was determined using dental growth layers. Carcasses were screened for ecto- and endoparasites and histopathological and microbiological investigations were conducted (Siebert et al., 2001; Siebert et al., 2017). Prevalence and level of parasitic infections were determined during
necropsy semi-quantitatively as none, mild, moderate or severe and associated lesions were preserved for subsequent histology (Lehnert et al., 2007; Siebert et al., 2007). During post-mortem investigation, the heart of the animal was separated from the lung, weighed, and opened starting with the atrium and continuing with the ventricle of the according side. Parasites were collected in water and cleaned from tissue before being preserved in 70% ethanol. Associated lesions were assessed macroscopically and archived in 10% buffered formalin for histology. Grey seal (n = 164) necropsy findings and archived tissue samples from 25 years of health monitoring seals on the coasts of Schleswig-Holstein were screened in the frame of a long-term study between 1996 and 2021. Two male grey seals exhibited unusual heart nematode infections. The carcass of the first case was frozen before necropsy, the carcass of the second case was necropsied freshly. Collected nematode specimens were identified with a stereomicroscope (Olympus SDX10 and SX61 with CD30, Olympus, Hamburg, Germany) based on their morphological characteristics (Leidenberger & Boström 2008). One adult female and one damaged heartworm specimen from the two different hosts were measured using CellSensEntry V3.2 software (Olympus, Hamburg, Germany). Because some isolated nematodes did not have unambiguous characteristics for parasite differentiation, species identification was achieved using gene sequence data. Genomic DNA was isolated from two specimens from the two grey seal individuals using a QIAamp DNA Micro Kit (Qiagen, Hilden, Germany). DNA concentrations and purity were determined using a Nanodrop 2000c (Thermo Scientific) spectrophotometer. Approximately 500bp of the mtDNA COI gene was amplified using oligonucleotide primers 5’-GGTCCCTGGGAGTAGCTGAAC-3’ (forward) and 5’-ATGATGGCCCCACACACAGAA -3’ (reverse) (Lehnert et al., 2015). Polymerase chain reactions were performed in 50 μL volume containing 25 μL MyTaq Red Mix, 2x (Bioline, Heidelberg, Germany), 1 μL of each primer (20 μM), 5 μL DNA template and distilled H₂O to fill the volume. Cycling conditions were initial denaturation at 95 ºC for 1 min,
followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 15 s and extension at 72 °C for 10 s. PCR products were visualised on a 2.0% agarose gel using SYBRSafe DNA gel stain (Invitrogen, Germany). PCR products were Sanger sequenced at Microsynth (Göttingen, Germany). The closest match to the sequence was determined using BLASTN on GenBank.

Results
Of 164 grey seals necropsied including the opening of the heart, six had samples of nematode infections in the heart. Four samples consisted of lungworm *O. circumlitus* *Otostrongylus circumlitus* (Crenosomatidae; Metastrongyloidea), but heartworm samples originating from two grey seals found along the German North Sea coast were identified as *A. spirocauda*, a filarial nematode belonging to the Onchocercidae (Filarioidea) (Fig. 1).

The first heartworm sample originated from a two-year-old male grey seal found in February 2018 on the island Heligoland. The animal was in state of preservation decomposition status grade 3 and in a good nutritional status with a body length of 147 cm. Due to predation the carcass displayed large wounds with missing skin, blubber and muscles and not all post mortem investigations could be performed. A moderate nematode infection of the left cardiac atrium and mild infections of the gastro-intestinal tract were recorded at necropsy. A focal moderate granulomatous and eosinophilic mural enteritis with intralesional nematode larvae was observed histologically. Microbiological culture revealed no specific pathogens.

The second heartworm sample originated from a seventeen-year-old male grey seal found alive on a beach of the island of Sylt in January 2020. Due to its poor health condition, it was mercy-killed. The animal was in a moderate nutritional status with a total length of 194 cm and a body weight of 114 kg. At necropsy one day later (decomposition status 1) a single nematode was collected from the heart. Severe infections with gastric anisakid nematodes and
Corynosoma spp. acanthocephalans in the small intestine as well as with respiratory mites Halarachne halichoeri were observed. In histology a moderate, granulomatous, mural gastritis with intralesional parasites as well as a marked eosinophilic and granulomatous enteritis accompanied by a similar lymphadenitis of the mesenteric lymph nodes were diagnosed. Additionally, pulmonary and gastric lymph nodes showed a mild follicular hyperplasia. A mild purulent valvular endocarditis was observed possibly indicating a septicemic origin. Microbiological investigation revealed the presence of Streptococcus equi subspecies zooepidemicus in nearly every organ sample. Only single colonies were detected in the pulmonary lymph node, intestine and intestinal lymph node, low growth rates in spleen, kidney, stomach and lung and moderate growth rate in the central nervous system (CNS). Strong growth of Clostridium perfringens was demonstrated in the intestine.

In both samples the nematode individuals appeared slender and whitish with firm rounded shape of the cuticula. Posterior body ends of males with characteristic helical tail and spicules for unambiguous morphological identification were not observed. In one sample the length of an intact female specimen was 9.2 cm, with a width of 0.5 mm. Other specimens were incomplete and in some instances entangled. The second sample contained only one damaged specimen of undetermined sex with a length of at least 12.3 cm and a width of 0.9 mm. The 474 bp long mtDNA sequence derived from the nematodes after PCR was 99.79% identical with A. spirocauda published sequence of the COI gene for cytochrome oxidase subunit 1 when blasted in GenBank (accession no.: HF583266.1).

Discussion

Nematodes collected from the heart of two male grey seals found along the German North Sea coast were molecularly identified as Acanthocheilonema spirocauda and comprise the first record of this species in grey seals during a decades-long monitoring of seal health along the
German North- and Baltic Sea coast. Heartworms are common in harbour seals and frequently infect individuals found stranded along the German North- and Baltic Sea coasts (Claussen et al., 1991; Lehnert et al., 2007, 2015) and adjacent waters (Lunneryd 1992). However, they were so far not reported from grey seals which share their habitat, diet preferences and haul-outs with harbor seals (Damseaux et al., 2021; Boyi et al., 2022; Brasseur 2017). The insect seal louse Echinophthirius (E.) horridus infects both harbour and grey seals and occur regularly on both species throughout the study area (Zimmermann & Nebel 1975; Lehnert et al., 2015) and in adjacent waters (Thompson et al., 1998; Morick et al., 2009). Although E. horridus is assumed to be an intermediate host and vector of the heartworm filariae (Geraci et al., 1981; Leidenberger et al., 2007; Lehnert et al., 2015), it was believed that heartworms do not infect grey seals (Measures et al., 1997; Leidenberger et al., 2007). A recent study found circumstantial evidence for A. spirocauda infecting grey seals by using a qPCR assay for heartworm infections, detecting a positive signal from a damaged nematode sampled from a seal carcass, assumed to be a grey seal but due to decomposition it was not possible to specify the host (Keroack et al., 2018). However, this is the first study actually reporting heartworm individuals found in the heart of grey seals at necropsy.

Lungworms belonging to the Metastrongyloidea usually infect the respiratory tract (Measures 2001) but are frequently found in the heart and surrounding blood vessels of seals at necropsy (Claussen et al., 1991) and can be mistaken for A. spirocauda, necessitating a thorough morphological or molecular identification. Otostrongylus (O.) (Crenosomatidae) and Parafilaroides (P.) gymnurus (Filaroididae) are found more seldom in grey seals compared to harbour seals, were prevalences of 70 % are common (Lehnert et al., 2007). The morphology of A. spirocauda (Onchocercidae) and two occurring lung nematode species O. circumlitus and P. gymnurus in grey seals is distinct. O. circumlitus lungworms are thicker, with their cuticula more crumpled and beige in coloration, P. gymnurus are significantly smaller and more fragile.
A. spirocauda. Especially adult male nematodes of the mentioned species display clear species-specific morphological traits in the posterior body end. Length and width measurements of an adult A. spirocauda specimen found in grey seals in this study corresponded with previous measurements of A. spirocauda found in harbour seals (Leidy 1858) also from the North Sea (Wipper 1974, van der Kamp 1987). However, it is challenging to differentiate damaged or larval nematode specimens. The emergence of molecular tools to differentiate between and speciate parasites without clear morphological traits enabled the identification of A. spirocauda with mitochondrial nucleotide data in this study. Based on the molecular data obtained, harbour and grey seals are infected by the same Acanthocheilonema species (A. spirocauda). Also, the lack of nucleotide difference between the grey and harbour seal heartworm shows that A. spirocauda of the same haplotype infects both species and the heartworm has not developed genetic adaptations to either species.

The low occurrence and intensity of A. spirocauda infections in grey seals in the study area over the last decades indicates a high species-specificity of heartworm as designated parasite of harbour seals. However, while seal populations have steadily increased over the last years (Olsen et al., 2018) and grey seals have recolonised the German North- and Baltic Sea after almost becoming extinct in the last century (Reijinders 2005), inter-species contacts and density-dependent infection patterns may influence the prevalence and intensity of A. spirocauda in both host species (Reckendorf et al., 2019). In the investigated grey seals heartworm infections were mild and moderate and probably did not substantially contribute to the cause of death or disease. However, both individuals had gastro-intestinal parasite infections, in one grey seal severe gastro-intestinal helminth and respiratory mite infections which debilitated the animal and probably contributed to its generalized infection. The detection of Streptococcus equi subspecies zooepidemicus in various organ samples together with the diagnosis of an endocarditis indicate that septicemia might have been the cause of
severe disease in this grey seal. The single heartworm encountered could also have caused mechanical alteration of the endothelium of the valve and provoked acute endocarditis. Although heartworms have been described to cause obstructions (Dunn & Wolke 1976; Stroud & Dailey 1978) in harbour seals, mechanical alterations like a perforation of the right atrium severely infected by *A. spirocauda* are scarce (Lehnert *et al.*, 2007).

The traumatic lesions in the first individual are assumedly caused by predation of another grey seal (van Neer *et al.*, 2020). Microbiological findings in the lung need to be judged with caution as the thorax was opened due to the predation wound and thoracic organs were probably contaminated from the outside. Inflammatory alterations in the intestine observed in histology were possibly caused by the parasitic infection. The parasites in the intestinal wall of both grey seals diagnosed in histology indicate the somatic migration of nematode larvae, however, it remains unclear if this may be heartworm, gastro-intestinal or other parasite species. Although heartworms seem to have mild pathogenicity in harbour seals – with mild infections and no significant impact on health apart from some cases (Dunn & Wolke 1976; Stroud & Dailey 1978; Conlogue *et al.*, 1980; Lehnert *et al.*, 2007) their pathogenicity in grey seals remains to be evaluated pending on more cases. On both grey seals no seal lice (*E. horridus*) were found, although they are suspected to be vectors of heartworm filariae (Geraci *et al.*, 1981). However, ectoparasites can leave their host after death, or may get lost during stranding, drifting or transport of the carcass, therefore introducing a bias into ectoparasite prevalence. It remains striking that although harbour and grey seals are closely related, share the same ecosystem and many resources, grey seals are not similarly affected by certain infectious diseases, e. g. phocine distemper virus (PDV), lung nematode (*Metastrongyloidea*) (Osinga *et al.*, 2012) and heartworm infections. Heterozygosity has been suggested to cause varying susceptibility to infectious disease (McCarthy *et al.* 2011) including lung nematodes (Rijks *et al.* 2008) within and among host species in natural harbour seal populations (Hoffman...
et al. 2014) when new approaches of uncovering heterozygosity fitness correlations for varying fitness within a population were investigated. Consequently heterozygosity could also be considered as possible factor explaining susceptibility to nematode infections in harbour and grey seals. Long term data sets and sample archives with preserved biological specimens and tissues collected over extended periods of time are especially valuable when investigating long-lived apex predators and effects of environmental change on their ecosystem (Reckendorf et al., 2019; Wood et al., 2022). Parasites can serve as important bio indicators for the ecology of their hosts, reflecting behaviour, diet and distribution (Marcogliese 2005; Hudson et al., 2006), and, in the case of grey seals potentially their reestablishment and subsequent richer biodiversity in the North Sea ecosystem (Marcogliese 2004). The intricate parasite-host relationships between heartworm and seals and the species-specificity of *A. spirocauda* as well as grey seal immune traits need to be investigated further.

**Acknowledgements.**

The authors thank all individuals who report and collect marine mammals in Germany, in particular the Schleswig-Holstein national park rangers and seal hunters, as well as all ITAW colleagues and assistants for their support during necropsies, especially Kornelia Schmidt and Miriam Hillmann. The investigations were partly funded by the Ministry of Energy Transition, Climate Protection, the Environment, and Nature (MEKUN S-H) and the National Park Service of Schleswig-Holstein.

**Author’s contributions.**

J.O.B., S.G., P.W., E. P.-B. and U.S. All authors have read and agreed to the submitted version of the manuscript.

Financial support.
The investigations were partly funded by the Ministry of Energy, Agriculture Environment and Rural Affairs, and the National Park Service of Schleswig-Holstein. This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

Competing interests
The authors declare there are no conflicts of interest.

Ethical standards.
All marine mammals in our study were found dead, died naturally or were mercy-killed based on animal welfare grounds and none of the animals were killed for the purpose of this study.
References


Conlogue, GJ, Ogden, JA and Foreyt, WJ (1980) Pediculosis and severe heartworm infection in a harbor seal. Veterinary medicine, small animal clinician 75(7), 1184-1187.


Hudson, PJ, Dobson, AP and Lafferty, KD (2006) Is a healthy ecosystem one that is rich in


Lunneryd, SG (1992). *Dipetalonema spirocauda* (Leidy) (Nematoda) and Corynosoma


**Morick, D, Osinga, N, Gruys, E and Harrus, S** (2009) Identification of a Bartonella species in the harbor seal (Phoca vitulina) and in seal lice (Echinoptiritus horridus). *Vector-Borne and Zoonotic Diseases* **9**(6), 751-753.


**Osinga, N, Ferdous, MS, Morick, D, Hartmann, MG, Ulloa, JA, Vedder, L, de Haes, HAU, Brakefield, PM, Osterhaus, AD and Kuiken, T** (2012) Patterns of stranding and mortality in common seals (Phoca vitulina) and grey seals (Halichoerus grypus) in The


Siebert, U, Rademaker, M, Ulrich, SA, Wohlse, P, Ronnenberg, K and Prenger-Berninghoff, E (2017) Bacterial microbiota in harbor seals (Phoca vitulina) from the


Figure 1. Heartworm *Acanthocheilonema spirocauda* with characteristic helical tail in adult male (a; inserted box) and *in-situ* right ventricle of seal heart (b; black box) found in grey seals (*Halichoerus grypus*) shown on haul-out on Helgoland (c).