Case Report:

A human case of outer ear canal infection with *Rhabditis* sp. nematodes

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Abstract

Here we report the first human case of an outer ear canal infection with a free-living nematode of the genus *Rhabditis*. Otomicroscopy revealed viable worms in the outer ear canal of a patient suffering from chronic otorrhea and hearing loss. The nematode was identified by microscopy and ITS-PCR.
Case Report

A 37-year-old male presented with purulent otorrhea from both ears for 8 weeks in a tertiary care center. Additionally, he reported of mild hearing loss since the beginning of symptomatic disease. Both otorrhea and hearing loss were more pronounced for the right ear, on which the patient had undergone mastoidectomy three years earlier due to chronic otitis media. The patient reported no other underlying disease or recent travel history, owned a pet dog as well as a pet cat and has been working in an industrial agriculture company.

Macroscopic inspection and visualization by otomicroscopy of the outer ear canal confirmed otorrhea and revealed tympanostomy tubes and a retraction of the tympanic membranes on both sides. The tuning fork test according to Weber lateralized left, the tuning fork test according to Rinne was bilaterally negative, no spontaneous nystagmus as a sign for disturbance of the vestibular system was detected. Pure tone audiometry presented a conductive hearing loss on both sides in combination with a mild sensorineural hearing loss of 20 dB hearing level (HL) in the middle frequencies on the right side. No other local or systemic signs of infection such as fever or enlarged cervical lymph nodes were detected.

Suspecting a chronic bacterial otitis media, local antibiotic ear drops containing ciprofloxacin (3 mg per mL) were prescribed. Bacterial swab cultures from the outer ear canal obtained prior to antibiotic therapy detected bacteria of the normal skin flora (coagulase-negative staphylococci, non-hemolytic streptococci and Corynebacterium spp.) as well as Alcaligenes faecalis, none of which are typically associated with otitis media. At that time, surgery was considered in the event of persistent clinical symptoms to exclude cholesteatoma. Cholesteatoma represents a benign proliferation of keratinizing squamous epithelium, which can lead to local tissue destruction and secondary chronic infection.

In a physical follow up examination four weeks later, no regression of clinical symptoms was noted. Surprisingly, otomicroscopy of both outer ear canals under 25-fold
magnification revealed a large number of viable, moving worm-like organisms [Fig. 1, arrow]. Both external ear canals were rinsed with saline solution and the lavage fluid was submitted for further parasitological examination. The patient was treated with repeated topical application of ethanol (dequaliniumchlorid 0.04 g, glycerin anhydricum, ethanol 90 % aa ad 20 g) twice a day. No blood tests for leukocytosis or eosinophilia were performed due to the absence of clinical symptoms of a general infection. Microscopy of the unstained lavage fluid revealed high numbers of viable, 500-1500 µm long nematode-like larvae morphologically similar to human pathogenic nematode species [Fig. 2A and B]. Lavage material and serum was sent for polymerase chain reaction (PCR)-based species identification and anti-nematode antibodies, respectively. Amplification of the ribosomal internal transcribed spacer 2 region was performed by PCR as recently described [1]. The amplified DNA was subsequently sequenced and a 874 nucleotide fragment was analyzed by BLAST search (blast.ncbi.nlm.nih.gov) revealing a 100% homology to free living nematodes of the genus Rhabditis (R. blumi, accession number DQ121436, 100% query coverage). The homology to the nearest sequence matches were 87% and 84% for Butlerius sp. and Pristionchus sp., respectively, two nematode genera, with only 32% and 30% query coverage. Serology detected low IgG-antibody titers against Ascaris lumbricoides (13 U/mL, normal value < 10 U/mL) using an in-house enzyme-linked immunosorbent assay (ELISA) with A. lumbricoides crude antigen extract and negative results for Dirofilaria immitis, Strongyloides stercoralis and Toxocara spp. To reveal the patient’s immunological response to the identified parasite, formalin-fixed Rhabditis sp. worm larvae from ear canal lavage were overlayed with 1:10 diluted patient serum and incubated for 30 min. at 37°C. Slides were washed in PBS 0.05% Tween 20, and incubated in 1:400 diluted goat anti-total human immunoglobulin (bioMérieux, fluoline H Cat# 75603) supplemented 1:10,000 diluted Evans Blue (bioMérieux, Cat# 75491) for another 30 min at 37°C. Clearly, the patient’s serum contained antibodies directed against Rhabditis nematode larvae whereas control serum obtained from a healthy
blood donor remained negative [Fig. 2C]. Stool samples from the patient contained no worm eggs or larvae.

A follow-up exam three weeks later revealed no signs of purulent infection of the external ear canal on either side and no viable nematode larvae were identified in ear canal lavage fluid. A cone beam computed tomography demonstrated the signs of a chronic bilateral mastoiditis and a post mastoidectomy status on the right side [Fig. 3]. Regular follow up exams during subsequent three months did not reveal any sign of recurrent disease.

The family *Rhabditiae* encompasses small free-living, saprophytic nematodes that live in soil and organic debris including also the well-studied model organism *Caenorhabditis elegans*. Adult male worms have an average length of 1.2 mm; female worms of 1.5 mm. They possess a buccal cavity and a rhabditiform oesophagus. Female worms show two ovaries and can be viviparous or oviparous. The life-cycle of most *Rhabditis* species is completed after several days and the nematode has occasionally been associated with human disease. *Rhabditis* larvae were isolated from human stool samples [2, 3, 4, 5, 6, 7, 8], urine samples [9, 10, 11, 12] and vaginal swabs [4]. The clinical relevance of the presence of the nematodes, however, has remained obscure in most cases and efforts to confirm the causative role of *Rhabditis* sp. e.g. by immunological tests or larger association studies or post-treatment analyses have not been published. The presence of *Rhabditis* sp. in the diagnostic material might therefore have been the result of environmental contamination. Meamar et al. [8] isolated *R. axei* from two imprisoned AIDS patients with watery diarrhea but no follow up analysis was performed and the causative role was not established. Ahn [5] reported the detection of *Rhabditis* sp. in stool samples of rural school children. Colonization, however, was temporary and no association with any clinical symptoms was reported. Also isolation from urine samples was not associated with any particular symptom or disease [9]. To date, no luminal infection of the outer ear canal with nematodes has been reported in humans.
However, *Rhabditis* is well known to cause external otitis in cows, mainly in older animals both during the rainy and dry season in tropical areas in Brazil and many African countries such as Zimbabwe or Tanzania [13].

The present case, to the best of our knowledge, represents the first report of a human ear canal infection with *Rhabditis* sp. Infection was associated with acute conductive hearing loss and purulent otorrhea and confirmed by the presence of anti-*Rhabditis* antibodies in the patient’s serum. The differential diagnosis consisted of a broad range of bacterial and fungal infectious agents and cholesteatoma [14, 15, 16]. The concomitant finding of *A. feacalis* in swab cultures of the ear canal was regarded as an accidental finding. It might, however, hint at a faecal route of contamination. However, the origin of the *Rhabditis* sp. infection has remained unclear. The patient reported neither travel to endemic areas of bovine external otitis nor intentional or unintentional occupational or leisure contact to animal feces. Also, the patient denied the application of any type of product to his ears or treatment with environmental material, which might have been a possible source of infection. His pet dog and pet cat were screened by routine veterinary microbiology but found negative for parasitic diseases. As nematode larvae from different genera are morphologically very similar, molecular identification of such organisms provides a potent diagnostic tool. The weak positive *Ascaris* serology results may reflect cross-reacting anti-*Rhabditis* antibodies; this result, however, may also originate from a past true ascariasis. The recommended treatment for *Rhabditis* infection in animals includes broad spectrum anthelmintic agents such as albendazole and ivermectin. An additional treatment option of local, non invasive infection consists in the local instillation of concentrated alcohol, which was effective in the treatment of the presented patient.

Together, we present the first human case of outer ear *Rhabditis* sp. infection. Although the incidence of parasitic infections of the ear in humans is certainly low, our report indicates that careful inspection of the ear canal by otomicroscopy and microscopic analysis of
lavage fluid provide valuable diagnostic information. Molecular diagnostic tools help to identify also uncommon pathogens.

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Figure 1: Otomicroscopy of the external auditory canal. Endoscopic view into the left outer ear canal onto the tympanic membrane with the central umbo (*) and the atypical cone of light in the upper half. Larvae can be depicted as undulatory light reflections (arrow).

Figure 2: Microscopic visualization of *Rhabditis spp.* larvae in ear canal lavage fluid. (A) Unstained microscopy of the ear canal lavage fluid. Bar, 50 µm. (B) Unstained (upper panel) and iodine stained (lower panel) male larvae. Bar, 100 µm. Description of the upper panel as follows: (1) stoma / buccal cavity; (2) esophagus, (3) esophageal bulb, (4) intestine, and (5) anus. (C) Indirect immunofluorescence staining of larvae in the lavage fluid using the patient’s serum (left panel, diluted 1:10) or serum obtained from a healthy blood donor (right panel, diluted 1:10).

Figure 3: Cone beam computed tomography of the patient’s temporal bones. Signs of a chronic bilateral mastoiditis as well as status post mastoidectomy on the right side (arrow) can be seen.
Teschner et al., Figure 3