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Molecular Identification of Zoonotic Tissue-Invasive Tapeworm Larvae Other than *Taenia solium* in Suspected Human Cysticercosis Cases

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Rarely, zoonotic *Taenia* species other than *Taenia solium* cause human cysticercosis. The larval stages are morphologically often indistinguishable. We therefore investigated 12 samples of suspected human cysticercosis cases at the molecular level and surprisingly identified one *Taenia crassiceps* and one *Taenia serialis* (coenurosis) infection, which were caused by tapeworm larvae normally infecting rodents and sheep via eggs released from foxes and dogs.

Human cysticercosis, a disease classified as neglected by the World Health Organization (WHO), is commonly caused by the larval stage of the pork tapeworm *Taenia solium* (1). The disease is characterized by single to multiple pea-sized developing cystic larvae (cysticerci) located in the subcutis, muscles, brain, and rarely, in the eye (2).

In the natural cycle, the adult strobilar tapeworm lives in the human intestine and produces high numbers of eggs that are fecally shed in the environment. When pigs ingest such eggs via contaminated food, larvae form cysts predominantly in the pig's musculature. The parasite's life cycle is closed when humans consume undercooked pork containing cysticerci that thereupon develop into strobilar tapeworms in the human intestine.

Classical human cysticercosis develops after accidental oral uptake of infective cestode ova shed fecally by intestinally infected humans.

Various other zoonotic tapeworm species, which are propagated in different predator-prey cycles, have been described as causes of human cysticercosis (3–6). Tapeworm larvae have a highly similar morphology. Histologically the species are often indistinguishable. Especially if only a limited amount of tissue is available for diagnostics, it may be impossible to find discriminatory features in the available section planes. Most cases of human cysticercosis infections due to non-*T. solium* larval tapeworms have been described in immunosuppressed individuals (5). However, by applying molecular methods, we recently identified *Taenia crassips* and *Taenia martis* as causes of cysticercosis in immunocompetent humans. The cysts were found in brain (3), subcutis (4, 5), and eye (6) and showed clinical and morphological features similar to those of classical cysticercosis. We therefore retrospectively analyzed FFPE samples from human cysticercosis cases by molecular methods in order to find out if non-*T. solium* cases were among them. All cases had been diagnosed morphologically as cysticercosis before, and *T. solium* had been assumed to be the etiological agent.

Twelve FFPE blocks from 12 individual patients (male-to-female ratio, 1:1, age 18 to 48 years at the time of diagnosis; Table 1) were sectioned in 3-μm and 10-μm slices for morphological and molecular analyses, respectively. As far as possible, one hematoxylin-and-eosin-stained and one Periodic acid-Schiff-stained section each was morphologically reanalyzed. In all investigated cases, cystic structures typical for tapeworm larvae were found, i.e., an eosinophilic tegument with characteristic spongiform parasite stroma, in most cases containing calcareous corpuscles. Serial sectioning was not possible in all cases due to material limitations, and a protoscolex was not seen in most samples. For molecular analyses, the tissue slices were deparaffinized, and DNA was extracted using the QIAamp DNA FFPE tissue kit (Qiagen, Hilden, Germany). PCRs targeting cestode cytochrome oxidase subunit I (*cox*) genes were performed (primers JB3 [TTTTTTGG GCATCCTGAGGTTTAT] and JB 4.5 [TAAAGAAAGAACATAA TGAAAATG]; 7). The resulting 388- to 424-bp amplicons were sequenced and compared to GenBank database entries using BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Successful PCR-amplification of cestode DNA was possible in five of the cases (42%; see Table 1). In the remaining cases, putative formalin-induced DNA cross-links of the comparably large ~400-bp PCR products likely prevented amplification. Of note, the tissue blocks from which parasite DNA detection was not possible were the oldest in the series we examined. Thus, in recently stored fixed-tissue material, molecular methods were suitable for the identification of tissue-invasive larval tapeworms. Three cases were molecularly confirmed to be *T. solium* cysticercosis cases (cases 10 to 12, each with 100% identical nucleotides to *T. solium* sequences deposited in GenBank). A comparison of *cox* sequences were already denoted as being of Asian-origin *T. solium* in cases 10 and 12 (highest similarity to *T. solium* sequences from India and Thailand, GenBank accession no. AB066489 and AB066487, re-

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TABLE 1 Characteristics of patients with histopathological diagnosis of cysticercosis[a]

Case no.	Yr	Age at time of diagnosis (yr)	Sex	Patient origin	Infected tissue type	Molecular diagnosis
1	2002	39	Male	Serbia	Brain	Negative
2	2003	35	Female	Germany	Muscle	Negative
3	2004	24	Female	India	Muscle	Negative
4	2005	29	Male	Germany	Muscle	Negative
5	2007	31	Female	Germany	Muscle	Negative
6	2008	30	Male	Nigeria	Subcutis	*T. serialis*
7	2008	32	Male	Germany	Muscle	Negative
8	2010	18	Female	Germany	Brain	Negative
9	2011	47	Male	Germany	Muscle	*T. crassiceps*
10	2012	42	Female	Germany	Brain	*T. solium*
11	2013	48	Male	South America	Muscle	*T. solium*
12	2015	39	Female	India	Muscle	*T. solium*

[a] In all cases, the presence of a single-cystic larval tapeworm was diagnosed, and thus the larval stage of *Taenia solium* was assumed.

spectively), and of South American origin in case 11 (highest similarity to sequences from Brazil, Mexico, and Peru GenBank accession no. AB066492, AB066490, and AF360865, respectively). Additionally performed *T. solium* genotype PCRs (8) confirmed these results (nucleotide substitutions in the *Ag2* nuclear DNA genes: GG—T substitution for the Asian genotype and TT—C substitution for the African/South American genotype after sequencing), which were in line with the patients' origins (cases 11 [South America] and 12 [India]) and travel history (case 10 [Asia]). Surprisingly, two zoonotic tapeworm species other than *T. solium* were identified in the series of human cases examined here: one case was identified as an infection caused by *Taenia serialis* (case 6; 99% identical nucleotides to a sequence from Kenya [GenBank accession no. AM503320]; see Fig. S1 in the supplemental material), and the other was due to *T. crassiceps* (case 9; 99% identical nucleotides to a sequence from the Netherlands [GenBank accession no. KF751223]; see Fig. S2 in the supplemental material). Consensus sequence alignments of the PCR amplification products of the five samples can be found in the supplemental material. The *T. serialis* infection was identified in an African patient from Nigeria with the cystic parasite larva located subcutaneously on the lower jaw. The *T. crassiceps* infection was found in a German patient with swelling of the neck, and the parasite was located in the sternocleidomastoid muscle. This patient's serum reacted weakly positive in a *T. solium* enzyme-linked immunosorbent assay (ELISA) but not in an immunoblot. Further serological data were available only for case 12 (*T. solium*), with a negative ELISA result and positive immunoblot result. The clinical follow-ups for cases 6, 9, 11, and 12 were unremarkable after successful surgery. No follow-up data on case 10 were available.

There were fewer cysticercosis cases caused by *T. solium* in our series than histologically expected and more caused by enzootic species (such as *T. crassiceps*) than anticipated that do not depend on human beings in the transmission/developmental cycle, arguably due to animal meat inspection and hygiene standards. An exact species diagnosis is probably of limited relevance as far as therapy is concerned (surgical excision and/or antiparasitic chemotherapy with praziquantel); however, for epidemiological reasons and future preventive measures, the correct species identification of the causative larval tapeworm is important. In "classical" *T. solium* cysticercosis, the natural reservoir host is the human being, and transmission can be interrupted by safe disposal of human feces containing infective helminth ova and by proper hand hygiene. In contrast, in the *T. serialis* coenurus and *T. crassiceps* cysticercosis cases diagnosed here, dogs and foxes are the natural final hosts, and transmission may be interrupted by regular deworming of canines.

T. crassiceps (case 9) has a different epidemiology than *T. solium*, and, unlike *T. solium*, *T. crassiceps* does not occur worldwide with a focus on the tropics. Rather, *T. crassiceps* is prevalent in northern temperate geographical zones; its predator-prey life cycle involves canines, such as foxes, and rodents (5). *T. crassiceps* cysticercosis also has a different prognosis than that of *T. solium* cysticercosis, as *T. crassiceps* larvae tend to spread locally in the tissues and are responsible for relapsing infections, owing to productive budding and hard-to-detect remaining small cysts after surgery (5). While *T. crassiceps* closely resembles *T. solium* larval morphology of a cysticercus (with the exception of external buddings that are sometimes not seen, depending on the section plane), *T. serialis* larval stages (case 6) form a coenurus. The coenurus is a multiprotoscolex larval stage that morphologically differs from the cysticercus, which develops only a single protoscolex. In contrast to cysticercosis, coenurosis is characterized by a large and more tissue-compressing cyst. *T. serialis* coenurosis occurs worldwide, with a focus on Africa, and the parasite is propagated in a predator-prey cycle that involves dogs and foxes as definitive hosts. Many animals may serve as intermediate hosts, including rabbits and other rodents, horses, cattle, sheep, and goats (9). When found in the central nervous system (CNS), larval cestode parasites may provoke seizures, ataxia, and further neurological symptoms (1–3). Radiologically, CNS *T. crassiceps* infection can be confused with a racemose cysticercus (3). Owing to their biological characteristics, larval tapeworm species have different disease prognoses (dissemination and relapses), which are especially important from a neurologist's point of view.

In conclusion, molecular identification attempts for morphologically similar larval cestode parasites found in human tissues may aid in establishing the correct diagnosis. Especially when larval cestodes are detected in specific anatomical locations, such as in the brain (3) or the eye (6), in patients irrespective of their origin or travel history, molecular examination should be attempted. Molecular methods are thus complementary tools to conventional histology, and by identifying the causative agent to the species level, the natural reservoir of the respective parasite can be elucidated. By applying molecular tests, more different tapeworm species responsible for human infection will likely be identified, and the epidemiology behind these infections will be more understandable. Ultimately, future infections can be prevented more effectively.

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