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ORIGINAL PAPER

Imported diphyllobothriasis in Switzerland: molecular evidence of *Diphyllobothrium dendriticum* (Nitsch, 1824)

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Abstract We report for the first time in Switzerland a clinical case because of *Diphyllobothrium dendriticum*, identified by molecular methods. We discuss the potential for this imported species to infect local intermediate hosts and thus to achieve autochthonous cyclic transmission.

Introduction

Diphyllobothriasis in Switzerland is a re-emerging disease, linked to the increasingly popular consumption of raw or undercooked infected fresh water fish. The Cestode *Diphyllobothrium latum* (L., 1758) is considered the causative parasite (Peduzzi and Boucher-Rodoni 2001; Terramocci et al. 2001; Dupouy-Camet and Peduzzi 2004). Its identification is usually based on basic morphological characteristics of tapeworm segments (proglottids) or eggs passed in the stool (Garcia 2001). Infection is often asymptomatic. Clinical signs include abdominal pain, diarrhoea and other digestive troubles, itch or megaloblastic anaemia. The parasitosis is most successfully treated with praziquantel (von Bonsdorff 1977; Bourée 1995). Tapeworms belonging to the genus *Diphyllobothrium* are distributed over a wide geographical range, and different species are related to cases of human infections: *D. nihonkaiense* in Asia (Ando et al. 2001), *D. klebanovskii* in Siberia (Curtis and Bylund 1991), *D. ursi* in Northern Canada and Alaska (Margolis et al. 1973; Curtis and Bylund 1991), *D. dalliae* in Alaska and Siberia and *D. dendriticum* in Northern Europe and Canada (Curtis and Bylund 1991). Because morphologic characteristics show a great inter-specific variability, morphological identification of proglottids and eggs is significantly hampered and does not always lead to a reliable taxonomic classification. Molecular genetic analyses can significantly contribute to the identification of samples with unclear taxonomic position (Gonzalez et al. 2006; Yera et al. 2006). In the present study, we identified by molecular means a morphologically atypical *Diphyllobothrium* specimen, collected from a Swiss patient.

Case report

A 59-year-old woman reported chronically relapsing courses of diarrhoea of unidentified origin for a period of approximately 2 years. On September 22, 2006, while defecating, she naturally shed a worm of approximately 15 cm in length. After the patient was given a single dose of 400 mg albendazole, the Institute of Parasitology of the University of Bern identified the specimen as *Diphyllobothrium* sp., based on the morphology of proglottids and eggs. The patient was switched to praziquantel (single dose of 10 mg/kg body weight). Symptoms rapidly disappeared,

Nucleotide sequences data reported in this paper are available in the EMBL, GenBank and DDJB databases under the accession numbers AM412738 and AM412739.

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and three stool samples collected 10, 15 and 20 days post-treatment were negative with regards to parasitological findings. A retrospective investigation to detect the route of infection revealed that the patient used to consume regularly crude wild salmon, Japanese sushi and fish carpaccio. In 1999, the patient spent 3 months in Canada and Alaska and reported having consumed fish on many occasions. In summer 2004, she spent 2 weeks in Norway, also frequently consuming fish.

The morphological characteristics of the adult tapeworm did not completely fit the standard description of *D. latum*. Parts of the proglottids were therefore sent to the Cantonal Institute of Microbiology (ICM) in Bellinzona, for confirmation of the identification by molecular analyses.

Materials and methods

At the ICM, the unusual-shaped proglottids and eggs underwent light microscopic examination (16×, 100× and 400× magnification) and genetic analyses.

Deoxyribonucleic acid (DNA) was extracted from 25 mg tissue (about two proglottids) using the QIAamp DNA Minikit (Tissue Protocol; Qiagen, Hombrechtikon, Switzerland). Polymerase chain reaction (PCR) analysis was done using *Taq* PCR Master Mix Kit (Qiagen) and specific primers for the amplification of 18S ribosomal ribonucleic acid (rRNA) and cytochrome *c* oxydase subunit I (COI) genes (Bowles et al. 1992; Mariaux 1998). DNA was amplified in a 50-µl reaction volume with final concentration of 2.5 U of *Taq* DNA polymerase, 1.5 mM of magnesium chloride, 200 µM of each deoxynucleotide triphosphate and 0.3 µM of each primer. Amplification was realised as follows: 94°C for 5 min; 40 cycles consisting of 30 s at 94°C, 40 s at 45°C and 1 min at 72°C; 10 min at 72°C. After manual correction, the nucleotide sequences obtained were edited using the program EditSeq™ (DNAStar). Their alignment was made with homologous 18S rRNA and COI gene sequences available in public databases. Reference sequences were *D. latum* (GenBank accession numbers AM712620 and AM712906), *D. nihonkaiense* (GenBank accession numbers AM408316, AM 412740, AM412559 and AM412560), *D. ditremum* (GenBank accession numbers DQ181944, DQ768195 and DQ768196), *D. dendriticum* (GenBank accession numbers DQ181945, DQ768193 and DQ768194), *D. stemmacephalum* (GenBank accession number AF124459), *D. pacificum* (GenBank accession numbers DQ925310 and AM747494; Wicht et al., unpublished). *Schistocephalus solidus* (Müller, 1776; Cestoda, Pseudophyllidea, Diphyllobothriidae; GenBank accession number AF124460), *Ligula intestinalis* (L., 1758; Cestoda, Pseudophyllidea, Diphyllobothriidae; GenBank accession number AF153910) and *Taenia solium* (L., 1758; Cestoda, Cyclophyllidea, Taeniidae; GenBank accession numbers DQ157224 and AF360865) were inserted as outgroups. The phylogenetic tree was produced using the neighbor-joining method (Kimura-2 parameters; bootstrap test for 500 replicates) using the program MEGA version 3.0 (Kumar et al. 2004).

Results

The few proglottids examined were longer than wide, with excentred utera; the overall helminth feature looked as if segments had been ‘stretched.’ Eggs showed quite variable sizes and measured 56.74±7.12×39.58±3.83 µm (n=18).

The partial 18S rRNA gene sequence of the sample (Proglottid, Bern AM412739) showed 99.8% similarity with both *D. dendriticum* (Nitsch, 1824) and *D. ditremum* (Creplin, 1827) reference sequences (Fig. 1). Partial COI gene sequence (Proglottid, Bern AM412738) showed 97.7% similarity with *D. dendriticum* reference sequences, and only 86.8 and 87.7% with *D. ditremum* references DQ768195 and DQ768196, respectively (Fig. 2).

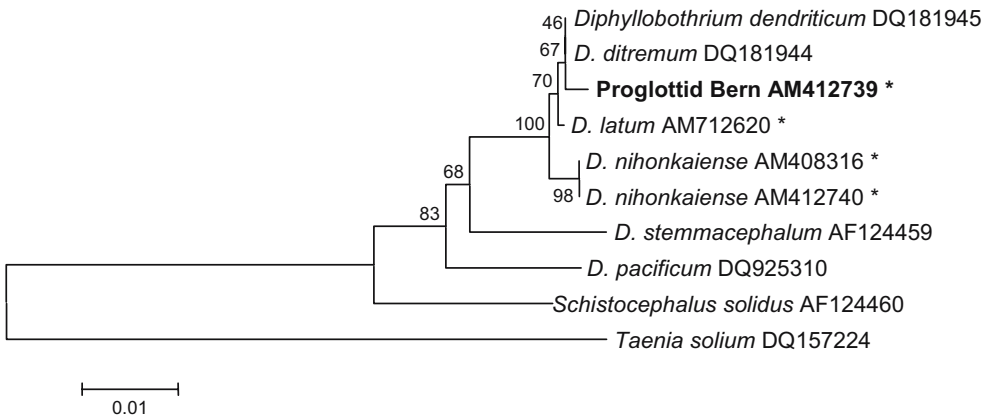

Fig. 1 Neighbor-joining tree on a 978-bp fragment of 18S ribosomal RNA gene (Kimura-2 parameters, bootstrap values for 500 replicates shown on nodes). *Asterisk*, *Diphyllobothrium* isolated from Swiss patient

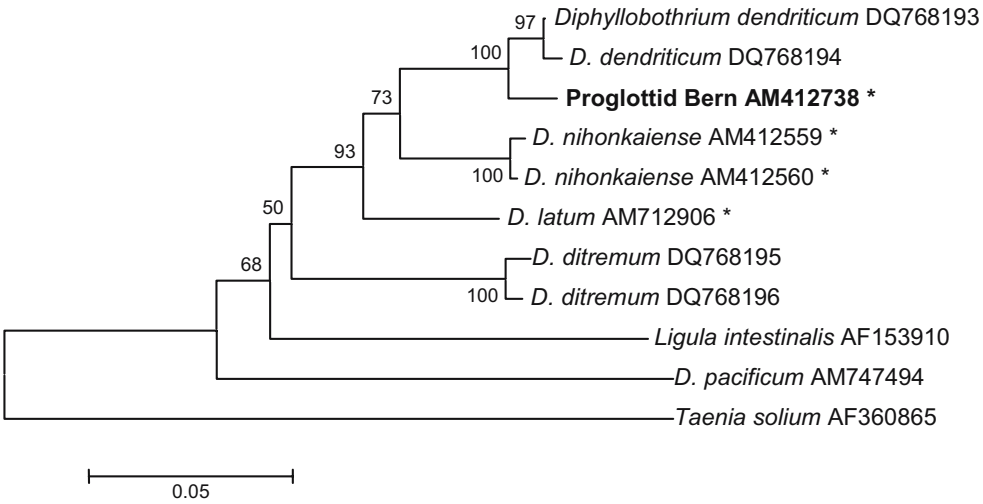

Fig. 2 Neighbor-joining tree on a 393-bp fragment of cytochrome *c* oxydase subunit I gene (Kimura-2 parameters; bootstrap values for 500 replicates shown on nodes). *Asterisk*, *Diphyllobothrium* isolated from Swiss patient

Discussion

We provide the first evidence of infection by *Diphyllobothrium dendriticum* diagnosed in Switzerland, identified by molecular methods. Because of the known geographic distribution of this parasite and to the long persistence of symptoms before diagnosis, a contamination during the journeys in Alaska, Canada or Norway is presumable. However, because proglottids usually pass in faeces only weeks or months after exposure, it cannot be excluded that infection occurred in Switzerland. Infections with such imported tapeworms seem to be linked to the increasing consumption of raw fish dishes (sushi, tartare, carpaccio), as was the case for *D. nihonkaiense* isolated from three patients in France and Switzerland, presumably infected from wild Pacific salmon (*Oncorhynchus keta*) imported from Canada or North America (Yera et al. 2006; Wicht et al. 2007). Infections with exotic species of *Diphyllobothrium* are probably more frequent in Switzerland and in other European countries than estimated. Diagnosis of diphyllobothriasis by physicians and laboratories requires therefore a detailed knowledge of the complex taxonomic problems, typical of this genus. Molecular methods could be useful for the accurate identification of *Diphyllobothrium* species in the presence of unusual symptomatology or atypical proglottids and eggs.

Parasites belonging to the genus *Diphyllobothrium* show sometimes a high colonization potential, as shown for *D. latum* and *D. dendriticum* (Torres et al. 2004). Eggs eliminated in stool can pass through sewage treatment plants and hatch in water basins. Thus, imported *Diphyllobothrium* species may become autochthonously transmitted by local competent intermediate hosts (copepods and fish). Copepods implicated in the life cycle of *D. dendriticum* include *Cyclops* and *Eudiaptomus* species (Rahkonen et al. 1996), some of which are present in Switzerland (*Eudiaptomus gracilis*, probably *Cyclops scutifer*). *D. dendriticum* is known to infect the rainbow trout (*Oncorhynchus mykiss*), the brown trout (*Salmo trutta*), the burbot (*Lota lota*) and coregonids (*Coregonus clupeaformis*, *C. albula*; Dick and Poole 1985; Rahkonen et al. 1996). These hosts are abundant in Switzerland, thus putatively allowing the cyclic transmission of *D. dendriticum*.

The availability of imported fish and relatively high rates of local fish contamination by *D. latum* (Nicoulaud et al. 2005; Wicht et al. 2006) emphasize the importance of appropriately cooking fish at 55°C for at least 5 min, or freezing it at −18°C for at least 24 h before consumption. These conditions should kill the larvae and thus help prevent infection by *Diphyllobothrium* tapeworms.

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