

A Human Case Report of *Diphyllobothrium latum* at Shanghai, China

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Abstract: Fish tapeworm (*Diphyllobothrium* species) are endemic in many parts of the world, the commonest species being *Diphyllobothrium latum*. Human are the main definitive host of *D. latum* and the majority of individuals harbouring the parasite are asymptomatic. In this report, researchers describe a case of *D. latum* infection founded on routine detection of eggs in fecal. After the whole cestode gotten, PCR Molecular Method had been done for identification.

Key words: *Diphyllobothrium latum*, eggs, cestode, PCR, parasite, China

INTRODUCTION

Human diphyllobothriosis, a worldwide fish-borne zoonosis, caused by tapeworms of the genus *Diphyllobothrium* Cobbold, 1858 (Cestoda: Diphyllobothriidea) with complex life cycles involved in two intermediate hosts (copepod or fish) and a definitive host (humans or other piscivorous mammals and aquatic birds) (Jeon *et al.*, 2009). Infection occurs through the consumption of raw or undercooked fish harboring plerocercoid larvae and often ignored until the excretion of proglottids in stools. Symptoms include various minor digestive problems occurring a few weeks after infection (mostly nausea, diarrhea and abdominal pain), less commonly, prolonged or heavy infections can result in a pernicious anemia (Lee *et al.*, 1989; Wu, 2005).

The identification of *Diphyllobothrium* tapeworms by physicians and medical laboratories is generally based on the morphological observation eggs and proglottids of adult worms (Jeon *et al.*, 2009). Morphoanatomical criteria allow identification to the genus level but are not reliable to assess species identity because the different taxa are extremely similar to one another and species differentiation relies on characteristics of the scolex or the genital apparatus observed on mature proglottids which are often unavailable during human infections (Stunkard, 1965). Thus, molecular methods have been recommended (Wicht *et al.*, 2010; Usmanova and Kazakov, 2010; Mercado *et al.*, 2010).

CASE REPORT

A 30 years old woman with a history of stomachache, abdominal distension, diarrhea and excreting white living proglottid, went to Xinrui Medical Centre to detect at Dec. 3rd, 2011. From 2008-2011, the patient had spent most of time in Shanghai and occasional vacation in Australia, France, Thailand, Japan, USA and some rural areas in China. During these 3 years, she had consumed raw fish (both sea fish and fresh water fish) in Japan and China. After 4 h by taking betel nut and pumpkin seed decoction and MgSO₄ solution for catharsis orally a whole cestode had been excreted successfully.

The cestode had been took to the laboratory of the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention to identify by morphology and molecular. The length of the cestode was nearly 5 m and the width was 1 cm with scolex as spoon like (Fig. 1). Moreover, there was also deep canalure in scolex and the width of mature proglottid was longer than the length evidently (Fig. 2). After dyed by coccinelling and microscopy of rosette-shape uterus like could be observed in the middle of their gravid proglottids. Eggs characteristic of *D. latum* with yellowish brown color and size of 61.0-65.3×41.7-46.1 μm and display a small knob or abopercular on the opposite end of a lid-like opening or operculum which may be in conspicuous (Fig. 3).

The DNA of mature proglottids has been extracted with SDS/proteinase K treatment, column-purified (Wizard® SV Genomic DNA Purification System, Promega)

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Table 1: Primers used in this report

Genes	Primers	Sequences	The length of product (bp)
<i>Cox1</i>	JB6 (common primer)	5'-GATAGTAAGGGTGTGA-3'	650
	JB5R (common primer)	5'-CAAGTATCRGCAAAATATTATCAAG-3'	
<i>Cox1</i>	DI/Dn-1805F (specific primer)	5'-CAGTGGGAATGGTGCTTGTAATGT-3'	428
	DI-2211R (specific primer)	5'-TAACCTTTACTTATACTACT-3'	



Fig. 1: Scolex of *Diphyllobothrium latum* (10 times)

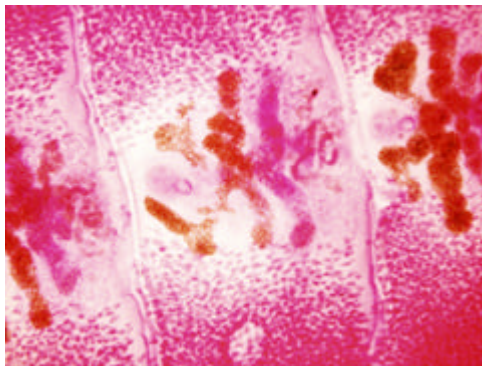


Fig. 2: Gravid proglottid of *Diphyllobothrium latum* (4 times)

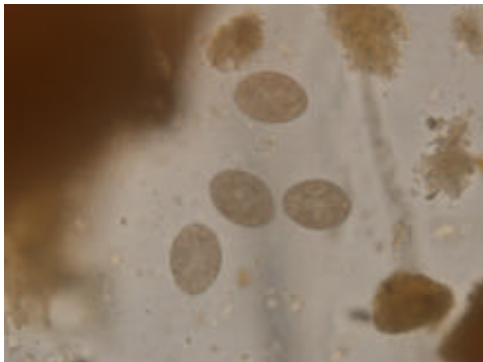


Fig. 3: Eggs of *Diphyllobothrium latum* (40 times)

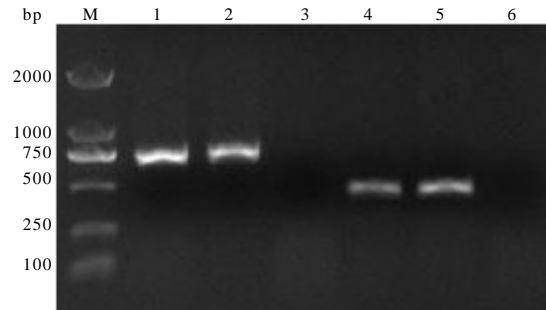


Fig. 4: Agarose gel electrophoresis of *pcox1* PCR products of representative *Diphyllobothrium latum* from patients. Lanes 1, 2 represent the PCR results of common primers, Lane 4, 5 represents the PCR results of specific primers. Lanes 3, 6 represents negative control. M represents a DNA size marker (ordinate values in bp)

(Ai *et al.*, 2010a, b). Then, the Polymerase Chain Reaction (PCR) Method also had been used in the identification by the primers to amplify part of *cox1* mitochondrial gene with common primers and specific primers (Table 1). PCR reactions (25 μ L) were performed in 2 mM of $MgCl_2$, 2.5 μ M of each primer, 2.5 μ L 10 \times rTaq buffer, 0.2 mM of each dNTPs, 1.25 U of rTaq DNA polymerase (TAKARA) and 1 μ L of DNA sample in a thermocycler (Biometra) under the following conditions: after an initial denaturation at 94°C for 5 min then, 94°C for 30 sec (denaturation); 55°C for 30 sec (for common primers) and 60°C (for specific primers) (annealing); 72°C for 30 sec (extension) for 35 cycles, followed by a final extension at 72°C for 5 min. Control samples without genomic DNA was included in each amplification run and in no case were amplicons detected in the controls. Each amplicon (3 μ L) was examined by agarose gel electrophoresis to validate amplification efficiency (Fig. 4). Positive amplicons purified and sequenced using an ABI 377 automated DNA sequencer (using BigDye Terminator Chemistry) employing the same primers used in the PCR.

Then, the sequences were blasted in NCBI and the results could be founded at Table 2 and 3. The results show that the cestode was identified as *D. latum*.

Table 2: The blasted result of the sequences producing significant alignments using the common primers

Accession	Description	Maximum score	Total score	Query coverage (%)	E-value	Maximum ident (%)	Links
AB511963.1	<i>Diphyllobothrium latum mitochondrial coxI</i> gene for cytochrome COX	1258	1258	98	0	99	-
AB510496.1	<i>Diphyllobothrium latum mitochondrial coxI</i> gene for cytochrome COX	1258	1258	98	0	99	-

Table 3: The blasted result of the sequences producing significant alignments using the specific primers

Accession	Description	Maximum score	Total score	Query coverage (%)	E-value	Maximum ident (%)
DO985706.1	<i>Diphyllobothrium latum</i> mitochondrion, complete genome	785	785	99	0	99
AB269325.1	<i>Diphyllobothrium latum</i> mitochondrial DNA, complete genome	774	774	99	0	99
AB268585.1	<i>Diphyllobothrium nihonkaiense</i> mitochondrial DNA, complete genome	547	547	99	2e-152	90
EF420138.1	<i>Diphyllobothrium nihonkaiense</i> mitochondrion, complete genome	536	536	94	4e-149	90

DISCUSSION

The adult *D. latum* parasites in the small intestine of dog, cat and other carnivore. Human was also the definitive host without obvious symptom or 40% may have low B12 levels but only 2% develop anacmia or nausea, vomit, diarrhea sometimes. This cestode mainly distributed in Europe, America and Asia. The infection rate was most high at Finland, Switzerland and Lithuania. Hitherto, there were 11 cases report about this disease with 9 cases inputting from former Soviet Union, American, Argentina and Japan. With the development and peoples living standard, raw and fresh fish will be more and more appreciated. So, the zoonic cestode disease must be reconstruction and prevention.

CONCLUSION

In this research, the case has been diagnosed not only by morphology of the parasite but also by PCR Method to amplify part of *cox1* gene and specific primers.

ACKNOWLEDGEMENTS

This research is supported in part by the Program for the International Development Research Centre, Canada (Project No. 105509-00001002), National S&T Major Program (Grant No. 2012ZX10004220, 2008ZX10004-001, 2008ZX10004-011, 2009ZX10004-302 and 2009ZX10004-201), National Key Technology R&D Program (Grant No. 2008BAI56B03) and the Open Funds of the State Key Laboratory of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences (Grant No. SKLVEB2009KFKT008, SKLVEB2010KFKT009, SKLVEB2011KFKT004 and SKLVEB2011KFKT010). The experiments comply with the current laws of the country in which the experiments were performed.

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