First Reported Diphyllobothriasis Outbreak in the State of São Paulo, Brazil, 2004-2005

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Unusual increase of diphillobothriasis in the city of São Paulo

Twenty one cases identified through laboratory-based active surveillance (morphology-based identification of *Diphyllobothrium* sp.)

Baseline - Two imported cases from 1998 to 2003.
Diphyllobothriasis

• Parasitic infection acquired by the ingestion of raw or undercooked fish

• Clinical Features
  – Most infections are asymptomatic (80%)
  – Diarrhea, pain or abdominal discomfort, weight loss, weakness, elimination of adult worm proglottids
  – Prolonged or heavy infection associated with anemia and obstruction of the bile duct or intestine

• Geographic Distribution
  – Europe, Asia, North America and South America
  – South American: Peru (*D. pacificum*), Argentina, and Chile (*D. latum*); autochthonous cases were never identified Brazil.
Diphyllobothrium latum

- **Definitive host:** humans (7) and fish-eating animals
- **Intermediate host:**
  - copepods of the genera *Cyclops* and *Diaptomus* (3-4)
  - predatory fish with life phase in maritime coast and/or freshwater (5-6)

Source: CDC DPDx Web site [http://www.dpd.cdc.gov/dpdx](http://www.dpd.cdc.gov/dpdx)
Objectives

- Describe the outbreak
- Identify the source of infection
- Prevention of new cases
Methods

- Cases series study
- Trace back investigation
- PCR and DNA sequencing analysis (confirmation of the parasite species and genotype).
Autochthonous Case Definition

- Presence of *Diphyllobothrium* sp. ova or strobila in feces, residents in the State of São Paulo without evidence of acquiring the disease outside Brazil, from March 2004 to December 2005.
Cases of Diphyllobothriasis
State of São Paulo, 2004-2005 (N = 54)

Source: DDTHA/CVE-SES/SP
Demographics Characteristics (N=54)

- **City of residence**
  - São Paulo 43 (80%)
  - Incidence rate = 4.3 cases/100,000 raw fish consumers

- **Age, years**
  - Median 30
  - Range 6-77

- **Gender**
  - Male 57%

- **Japanese descendants** 13%

- **Social-economic status** High

*Approximately 3% of Sao Paulo state population are Japanese descendants.*
## Characteristics of Illness Among Cases (N=54)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>33</td>
<td>61</td>
</tr>
<tr>
<td>Abdominal pain/Discomfort</td>
<td>24</td>
<td>44</td>
</tr>
<tr>
<td>Flatulence</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td>Anemia</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Hospitalized</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Symptomatic</td>
<td>43</td>
<td>80.0</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>11</td>
<td>20.0</td>
</tr>
</tbody>
</table>
## Disease x Exposition: Risk factors identified (N=54 cases)

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fish consumed (method of preparation)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Raw (sushi/sashimi)</td>
<td>54</td>
<td>100</td>
</tr>
<tr>
<td>- Raw and undercooked</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>- Raw and smoked</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td><strong>Local of consumption</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Japanese restaurants</td>
<td>50</td>
<td>93</td>
</tr>
<tr>
<td>- At home</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td><strong>Species of raw fish consumed</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Salmon and other species</td>
<td>28</td>
<td>52</td>
</tr>
<tr>
<td>- Only salmon</td>
<td>26</td>
<td>48</td>
</tr>
<tr>
<td>- Never had traveled outside Brazil</td>
<td>21</td>
<td>81</td>
</tr>
</tbody>
</table>
Trace Backs

- Commercial establishments acquired salmon from a Food Distribution Center (CEAGESP) in São Paulo or directly from importers.

- Five importers from Chile supplied all commercial source of fish.

- All salmon originated from fish farms located in Puerto Montt, Southern Chile.

- Since 2003 Brazil increased the importation of fresh salmon - 12,000 ton/year, imported exclusively from Chile (Source: Brazilian Department of Agriculture).
Molecular analysis - 18S rRNA

• DNA was extracted from *Diphyllobothrium* proglottids obtained from three patients diagnosed during the outbreak.

• Full length 18S rRNA gene was amplified by PCR, cloned and sequenced.

• Sequences from the three Sao Paulo isolates were 99.8% similar to a *D. latum* 18S rRNA sequence from GenBank.

Note: *D. latum* 18S rRNA sequences from Sao Paulo isolates were deposited in GenBank under accession numbers DQ316793, DQ316794, DQ316795, DQ316796.
Molecular analysis - ITS1 and ITS2

18S rRNA  5.8S  28S rRNA
DIPHYF7*# 1771 - 1792
DIPHY28SR2*# 1 - 22

PCR product including ITS1, ITS2 and 5.8S rRNA ~ 1600 bp

* Primers used for PCR reactions (F7/28SR2 samples # 1, 2 and 3)
# Primers used for Sequencing reactions

→ Forward primer: Designed on the basis of *Diphyllobothrium stemmacephalum* 18S small subunit ribosomal RNA gene (GenBank entry AF124459)

← Reverse primer: Designed on the basis of *Diphyllobothrium latum* 28S ribosomal RNA gene (GenBank entry AF004719)
Molecular analysis - ITS 1 and ITS2

The three Sao Paulo isolates were 99.5% similar among them in ITS1 and ITS2.

ITS1 and ITS2 sequences from Sao Paulo isolates were distinct at 16 nucleotide positions (insertions/deletions, transitions and transversions were observed) from a *D. latum* isolated in the U.S (a case of infection diagnosed at CDC). The similarity between these isolates was of approximately 98%.

Note: No ITS1 or ITS2 sequencing data for *Diphyllobothrium* sp. is available in GenBank
Conclusions

• The outbreak was caused by imported fresh salmon.

• Sequences from ITS1 and ITS2 were useful to establish an epidemiologic link among isolates from three outbreak cases. Analysis of ITS1 and ITS2 from several *Diphyllobothrium* sp. isolates is needed for further validation of these markers as molecular epidemiology tools.

• Additional epidemiologic studies are developed to monitoring this disease in São Paulo.
Steps taken

- Release of updated sanitary regulation on fish consumption
- Media campaign aiming at informing physicians about general aspects of the disease, including diagnosis and treatment
- Sanitary measures for restaurants and other commercial establishments
- Implementation of molecular methods to study *Diphyllobothrium* sp. isolates in the IAL reference diagnostics laboratory
Acknowledgements

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