

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

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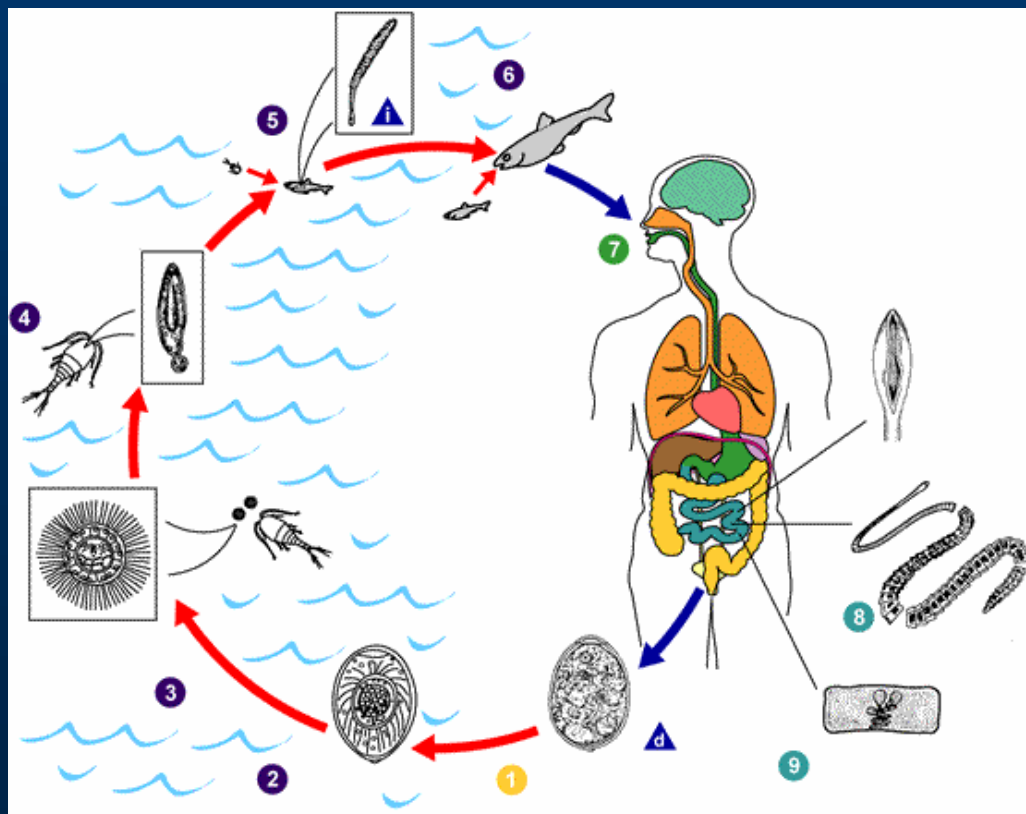
- Unusual increase of diphyllobothriasis in the city of São Paulo
- Twenty one cases identified through laboratory-based active surveillance (morphology-based identification of *Diphyllobothrium* sp.)
- Baseline - Two imported cases from 1998 to 2003.

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

D. latum, *D. pacificum*,
D. klebanovskii,
D. nihonkaiense



Source: CDC DPDx Web site
(<http://www.dpd.cdc.gov/dpdx>)

- Life cycle:
 - 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)

Objectives

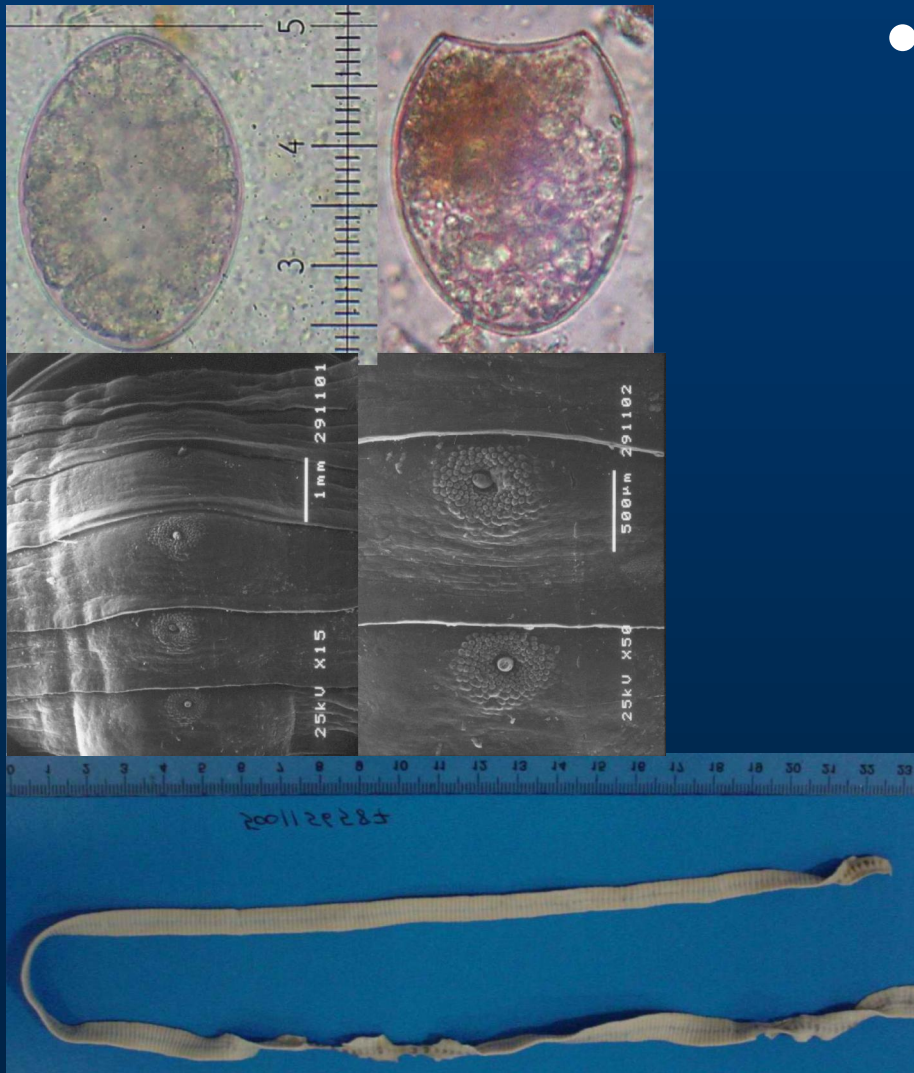
- **Describe the outbreak**
- **Identify the source of infection**
- **Prevention of new cases**

Methods

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

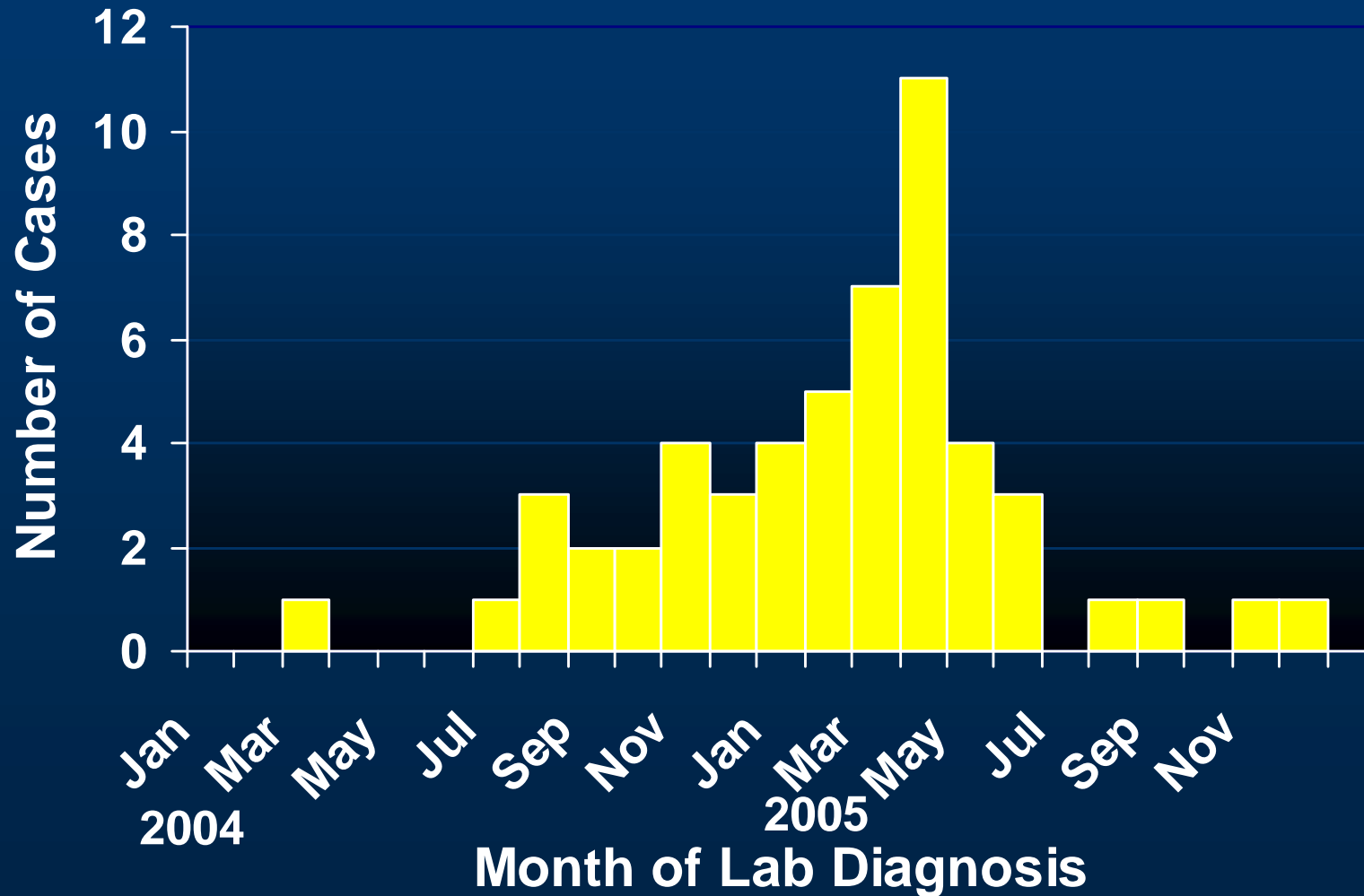
Autochthonous Case Definition

- 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.



Mello-Sampaio et al. *Diphyllobothriasis, Brazil*. *Emerg Infect Dis* 2005 Oct.
Available from <http://www.cdc.gov/ncidod/EID/vol11no10/05-0377.htm>

Cases of Diphyllobotriasis 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)

Characteristic	N	%
Diarrhea	33	61
Abdominal pain/Discomfort	24	44
Flatulence	12	22
Anemia	7	13
Hospitalized	7	13
Symptomatic	43	80.0
Asymptomatic	11	20.0

Disease x Exposition: Risk factors identified (N=54 cases)

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

Trace Backs

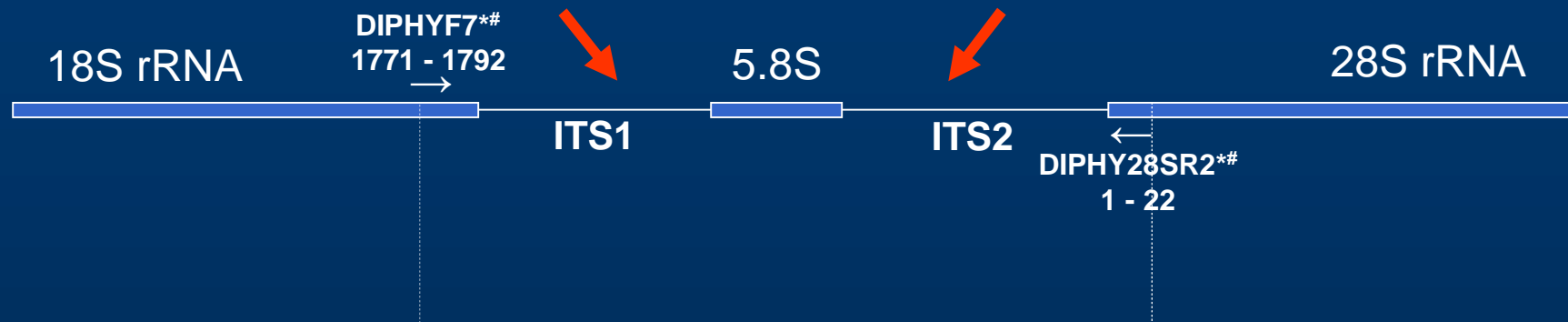
- **Commercial establishments acquired salmon from a Food Distribution Center (CEAGESP) in São Paulo or directly from importers**
- **Five importers from Chile supplied all commercial source of fish**
- **All salmon originated from fish farms located in Puerto Montt, Southern Chile**
- **Since 2003 Brazil 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 *Diphyllbothrium* 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



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 *Diphyllbothrium stemmacephalum* 18S small subunit ribosomal RNA gene (GenBank entry AF124459)

← Reverse primer: Designed on the basis of *Diphyllbothrium 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 *Diphyllbothrium* 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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