Resurgence of human bothriocephalosis (*Diphyllobothrium latum*) in the subalpine lake region

Raffaele PEDUZZI[1]* and Renata BOUCHER-RODONI[2]

[1]Istituto Batteriosierologico Cantonale, Via G. Buffi 6, 6904 Lugano, Switzerland
[2]Biologie des Invertébrés marins (BIMM, MNHN), UPESA 8044 CNRS, 55 rue Buffon, 75005 Paris, France
*e-mail corresponding author: raffaele-peduzzi@ti.ch

ABSTRACT

Human bothriocephalosis is once again being found in various catchment basins in the subalpine region, including Lago Maggiore and Lac Léman, which however are not isolated cases. Domestic animals are thought to be responsible for the survival of the parasite during the period when no human cases were reported. The new phenomenon of eating raw or poorly cooked fish is responsible for the resurgence of human bothriocephalosis, which affects various lake districts in Europe. This habit of eating raw fish might lead to the resurgence of a much more dangerous human parasitosis, transmitted in a similar way: infestation by Anisakis.

Key words: Bothriocephalosis, Diphyllobothrium*, Subalpine region*

1. INTRODUCTION

Water is a vehicle for many infectious and parasitic agents. Changes in social, cultural and agricultural habits have been accompanied by changes in the health risks associated with water. Human infection by *Diphyllobothrium latum* (Cestoda: Pseudophyllidaea), the broad tapeworm, is caused by the ingestion of raw or poorly cooked fish containing plerocercoid larvae. The infestation can occur only if the larvae are still alive after their ingestion. Maturation lasts for 5 to 6 weeks before egg production; the adult worm can be over 10 meters long and contains 2000-3000 proglottids. Diagnosis is usually based on the identification of characteristic eggs or proglottids in human faeces. The life cycle of the parasite includes two intermediate hosts (copepod and fish, Fig. 1). The occurrence of this parasite was originally restricted to the «old world», i.e. in lakes of various European countries such as Sweden, Finland, Russia, Poland, Rumania, Austria, Scotland, Ireland (O'Fel 1992; Dorucu *et al*. 1995; Byrne *et al*. 2000). Bothriocephalosis was apparently already present during pile-dwelling times: eggs have been found in human coprolites from Neolithic sites, in Jura (Bouchet 1997). Its introduction into the United States is recent. Cases have also been reported lately from Argentina and Chile (Semenas & Ubeda 1997) and from Asiatic countries like Japan and Korea, where the consumption of raw fish is common (Chung *et al*. 1997).

It used to be thought that this parasitosis had disappeared from many European and American regions. However, this paper reports that *Diphyllobothrium* is still present in fish samples from Lago Maggiore and Lago d'Orta, and that human infestation has been increasing in recent years in the subalpine region.
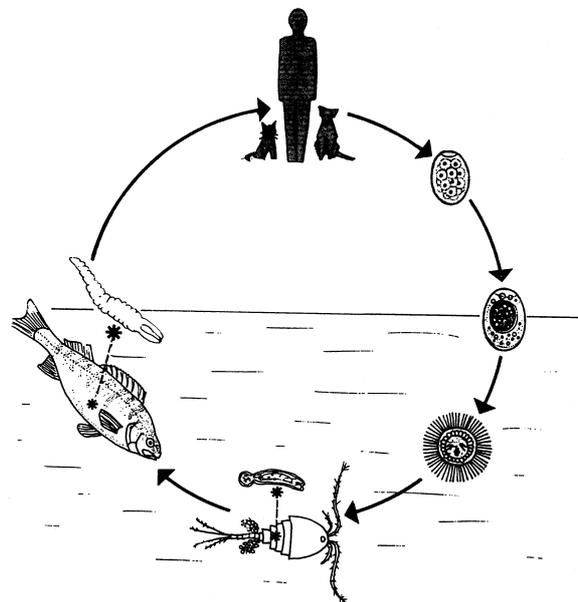

Fig. 1. Life cycle of *Diphyllobothrium latum* in Lago Maggiore. Two intermediate hosts are involved, a copepod *Cyclops* and a fish (mainly *Perca fluviatilis* but also *Esox lucius* and *Lota lota*). Plerocercoid larvae are found in fish muscle as well as in its body cavity.

2. MATERIAL AND METHODS

2.1. Human faeces analyses

Over 35,000 samples of human faeces were analysed in the last ten years, and tested for helminth infestation. To detect the presence of *Diphyllobothrium*, the samples were homogenised by a stomaker and by a diphasic technique derived from the Telemans-Rivas method (Bourée 1989). After centrifugation, *Diphyllobothrium* eggs were examined under a light microscope.
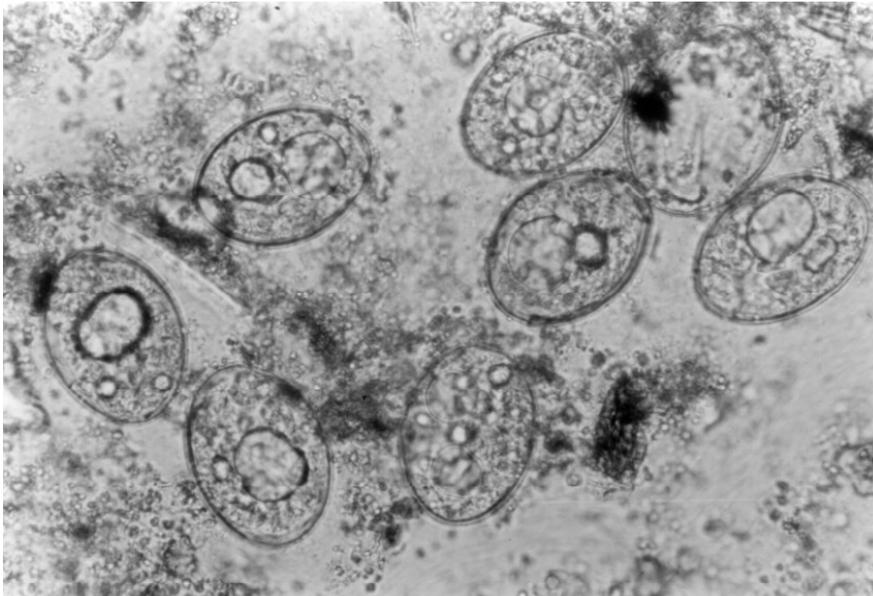
Fig. 2. Microscope view of *Diphyllobothrium latum eggs*, showing the high density of their release, which can reach one million eggs a day. Size of a single egg: 50 × 70 μm.
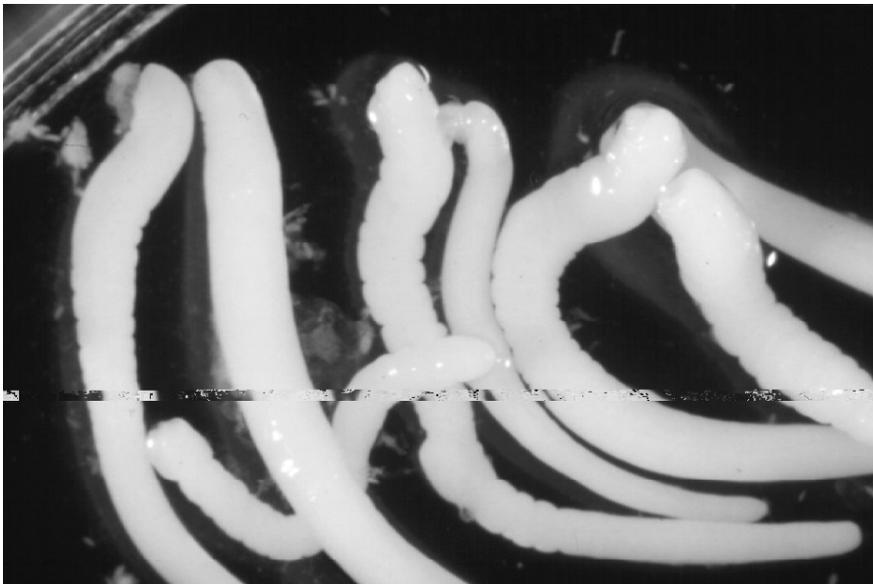
Fig. 3. Fish muscle (*Perca fluviatilis*) infestation by a large number of plerocercoid larvae of *Diphyllobothrium latum*. Scale 8×.

Fig. 4. Scanning electron microscope view of a plerocercoid larva of *Diphyllobothrium latum*: detail of buccal apparatus. Scale 45×.

2.2. Fish examination

Muscle samples of *Perca fluviatilis* were collected in various lakes from the subalpine region along the Swiss-Italian border (Tab. 1), and tested for the presence of parasite larvae by anatomic and microscopic observation.

Tab. 1. Presence of *Diphyllobothrium latum* larvae in the muscles of perch (*Perca fluviatilis*) from five Italian and Swiss-Italian subalpine lakes. I = Italy; CH = Switzerland.

Lake	N° fish	N° *D.latum* larvae	% infec.
Lugano (I-CH)	107	0	0
Varese (I)	35	0	0
Mergozzo (I)	50	0	0
Maggiore (I-CH)	309	24	7.8
Orta (I)	15	5	33.3

2.3. Electron microscope analyses

The samples were dehydrated in a series of ethanol baths of increasing concentration (20 to 100%, 10 min. per bath) followed by a last bath in pure acetone, before being transferred to the CO_2 critical point dryer CPD-030 (Balzers). Gold shadowing was performed in a sputter coater SDC-050 (Balzers), before observation with a scanning electron microscope (ISI SS40).

Tab. 2. Species and frequency of helminth isolated from human faeces from 1982 to 1999, out of 37,800 analyses.

Species	Number of cases
Trichuris trichiura	138
Taenia sp.	127
Enterobius vermicularis	66
Ascaris lumbricoides	44
Diphyllobothrium latum	31
Ancylostoma sp.	31
Hymenolepis sp.	12
Strongyloides stercoralis	9
Dicrocoelium sp.	8
Schistosoma haematobium (urine)	5
Trichostrongylus sp.	1
Clonorchis sinensis	1
Total number of cases	476

3. RESULTS AND DISCUSSION

In the Swiss-Italian subalpine region, considered in this study, *Diphyllobothrium latum* infestation in man had been presumed extinct since 1970. However, a first new case was identified from human faeces in 1983, and since then 31 other cases have been reported in the local population (anamnesis excludes infection from other sources), indicating that the parasitosis is now in prog??ress (Peduzzi 1990; Fig. 2). At present, in the geographical area considered, infestation by *Diphyllobothrium* is the most frequent in man after *Taenia*, *Trichuris* and *Ascaris* (Peduzzi & De Rossa 1999; Tab. 2). The percentage of *Diphyllobothrium* eggs varied from 0.3 to 0.4% of all the samples analysed in this study, a much higher value than the 0.01% frequency reported for this parasite by the CDC, Atlanta, on the basis of epidemiological data relating to USA (Stürchler 1986).

Tab. 3. Presence of *Diphyllobothrium latum* larvae in the muscles of perch (*Perca fluviatilis*) from Lago Maggiore. I = Italy ; CH = Switzerland.

Fishing zones	N° samples	N° *D.l.* larvae	% infec.
Riva Piana-Muralto (CH)	29	4	13.8
Bolle Magadino (CH)	15	1	6.7
San Nazzaro (CH)	11	0	0.0
Ranzo (CH)	12	0	0.0
Foce Ticino Verzasca (CH)	22	3	13.6
Vira Gambarogno (CH)	28	3	10.7
Laveno (I)	80	5	6.2
Angera (I)	50	1	2.0
Arona (I)	15	2	13.3
Stresa (I)	15	3	20.0
Baveno (I)	16	0	0.0
Verbania (I)	16	2	12.5
TOTAL	309	24	7.8

As far as fish infestation is concerned, plerocercoid larvae are found in fish muscle (Figs 3 and 4). A high infestation rate is recorded in perch (*Perca fluviatilis*) from many fishing areas of Lago Maggiore (Bonini *et al*. 1998; Tab. 3); the infestation is also in progress in other subalpine lakes, such as the Franco-Swiss lac Léman (Peduzzi *et al*. 1994). However, the resurgence of human bothriocephalosis is not restricted to these two major lakes, but involves most of the catchment basins in the entire subalpine region (Tab. 4). In addition, Golay & Mariaux (1995) reported 73 cases of bothriocephalosis from various areas in Switzerland, including the Léman region (Geneva, Vaud), but also Neuchâtel, Fribourg and Bern.

Tab. 4. Cases of human bothriocephalosis reported from various laboratories (1990-1995). I=Italy; CH=Switzerland; F=France.

Lake	Number of cases
L. Léman (F-CH)	24
L. Maggiore (I-CH)	28
L. Como (I)	1
L. Iseo (I)	8
L. Garda (I)	12

These results show that the parasite cycle was still active even when the disease did not appear in humans. Man is not the only ultimate host, so that the cycle may have been maintained through domestic animals (mainly cats and dogs). Human beings used to be protected by the habit of cooking fish at high temperatures, since the

most effective means of killing the parasite is by heating or freezing (Tab. 5).

Tab. 5. Survival of plerocercoid larvae to freezing and heating.

Temperature	Survival time
-10°C	1-7 days
+4°C	72 hours
+50°C	10 min.

Changes in eating habits, i.e. the consumption of raw or poorly cooked fish, popularised by specialised restaurants, have caused infestation in humans to reappear in areas where it was considered extinct. This alerts us to the risk of another infection, in which the process of contamination is similar but which is much more dangerous : infestation by *Anisakis*. In any case, eating raw or poorly cooked fish from lakes or sea can increase the risk of food-transmitted dangerous parasitosis in man.

REFERENCES

Bonini, P., S. Montorfani, R. Peduzzi & P. Renon. 1998. Situazione della plerocercosi nei laghi insubrici italo-svizzeri. Valutazione del potenziale zoonosico. *Obiettivi & Documenti Veterinari*, Bologna, 4: 65-72.

Borroni, I. & E. Grimaldi. 1974. Ecologia dell'infestione da larve plerocercoidi di *Diphyllobothrium latum* (Cestoda: Pseudophyllidaea) a carico delle specie ittiche recettive del Lago Maggiore. *Riv. Parassitol.*, 35(4): 261-276.

Bouchet, F. 1997. Helminth eggs : trace elements of neolithic and paleolithic parasitosis on French sites. *C.R. Séances Soc. Biol. Fil.*, 191(4) : 529-536 (in French).

Bourée, P. 1989. *Dictionnaire de parasitologie*. Editions Ellipses, Paris: 126 pp.

Byrne, C.J., C. Grey, C. Holland & R. Polle. 2000. Parasite community similarity between four Irish lakes. *J. Helminthol.*, 74(4): 301-305.

Chung, P.R., W.M. Sohn, Y. Jung, S.H. Pai & M.S. Nam. 1997. Five human cases of *Diphyllobothrium latum* infection through eating raw flesh of redlip mullet, *Liza haemotocheila*. *Korean J. Parasitol.*, 35(4): 283-289 (in Korean).

Dorucu, M., D.W. Crompton, F.A. Huntingford & D.E. Walters. 1995. The ecology of endoparasitic helminth infections of brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) in Scotland. *Folia Parasitol.* (Praha), 42(1): 29-35.

Golay, M. & J. Mariaux. 1995. Situation de *Diphyllobothrium latum* L. 1758 (Cestoda: Pseudophyllidea) dans quatre lacs du plateau suisse. *Bull. Soc. Neuch. Sci. Nat.*, 118: 79-86.

O'Fel, A. 1992. *Parasitologie et mycologie*. La Madeleine Editions C. et R.: 369 pp.

Peduzzi, R. 1990. Résurgence de la botriocephalose (parasitose à *Diphyllobothrium latum*) dans la région du Lac Majeur. Signalement de 18 cas chez l'homme. *Médecine & Maladies Inf.*, Paris, 20(10): 493-497.

Peduzzi, R., A. Demarta & M. Tonolla. 1994. Problèmes hygiéniques des lacs subalpins: influences des stations d'épuration et réapparition du *Diphyllobothrium latum*. In: *ACTES Quatrième conférence Internationale des Limnologues d'Expression Française*, Marrakech.

Peduzzi, R. & R. De Rossa. 1999. I parassiti più frequenti nella pratica analitica quotidiana. *Tribuna Medica Ticinese*, 573-576.

Semenas, L. & C. Ubeda. 1997. Human diphyllobothriasis in Patagonia, Argentina. *Rev. Salude Publica*, 31(3): 302-307 (in Spanish).

Stürchler, D. 1986. Intestinale Parasitosen. *Schweizerische Medizinische Wochenschrift.*, 116: 804-810.