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Prevalence of the broad tapeworm *Diphyllobothrium latum* in perch (*Perca fluviatilis*) and analysis of abiotic factors influencing its occurrence in Lake Lario (Como, Italy)

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Abstract

Diphyllobothriosis affects man and mammals as definitive hosts, as well as several fish species as intermediate or paratenic hosts. In Italy, in the last decade, *Diphyllobothrium latum* (Cestoda, Diphyllobothriidea) has shown a comeback around the shores of Lake Lario (Como), with several cases diagnosed from 2001. Anamnestic data constantly reported the consumption of raw or undercooked fish products in restaurants or local markets. In this study, we carried out a parasitological survey of perch caught in Lake Lario. Between 2005 and 2007, 609 perches (*Perca fluviatilis*) were sampled from 5 sites located along the three major branches of the lake and examined for the presence of plerocercoids larvae in the fillets. Data on abiotic factors in the same sampling areas were registered. The mean infection rate of perch was 30%, a value much higher than those observed in other sub-alpine lakes. The data collected indicate that the concentration of total phosphorus could influence the prevalence by *D. latum*, which seems to be higher in basins with low trophic conditions. Further studies need to be assessed to confirm the relation between prevalence and abiotic factors and to clarify the principal aspects that allow the endemic status of diphyllobothriosis.

Introduction

Human diphyllobothriosis is a widespread fish-borne zoonosis caused by tapeworms of the genus *Diphyllobothrium*. In the past century, for many years this disease seemed to be strongly reduced or disappeared in many European countries, but since the early '80s it has shown a comeback in some sub-alpine areas, particularly on the shores of the great lakes in Switzerland, France and Northern Italy (Peduzzi, 1990; Dupouy-Camet & Peduzzi, 2004).

In the Insubrian region, the prevalence of *D. latum* showed an increase both in humans (definitive hosts) and in fish (intermediate hosts) of several lakes (Magatelli, 1987; Chiodera et al., 1987; Peduzzi et al., 1992; Terramocci et al., 2001; Dupouy-Camet & Peduzzi, 2004). Anamnestic data could relate it to eating raw or undercooked local fish products, particularly perch (*Perca fluviatilis*) and whitefish (*Coregonus lavaretus*). Parasitological surveys showed the presence of

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plerocercoid larvae also in other fish species, such as pike (*Esox lucius*), burbot (*Lota lota*), charr (*Salvelinus alpinus*) and rainbow trout (*Salmo trutta*) as previously reported in the literature (Borroni e Grimaldi, 1973; Morishita et al., 1973; Woo, 1996). Infection rates of perch in Insubrian basins vary considerably. In the lakes Varese, Mergozzo and Ceresio (Lugano), fish seem not to be infected, whilst between 2005 and 2008 up to 14% individuals were infected in Lake Maggiore, and up to 33% in Lake Orta (Bonini et al., 1998; Peduzzi & Boucher-Rodoni, 2001; Wicht et al., 2009). In the Lake Lario (Como) the last surveys were carried out more than 30 years ago, reporting 16% of infected perches (Borroni & Grimaldi, 1973; 1974). Only few surveys were carried out in lakes Garda and Iseo, reporting very low levels of prevalence (0.4%) for Garda and 25% for fish of Lake Iseo (Parona, 1887; Scolari, 1955; Borroni & Grimaldi, 1973).

According to a recent study carried out in some lakes located in endemic sub-alpine regions, abiotic factors such as temperature, oxygen concentration and total phosphorus seem to play a role in maintaining infection in perch by *D. latum* (Wicht et al., 2009). The parasite was present in basins characterized by oligo-mesotrophic conditions (total phosphorus range: 10-20 µl l-1), summer temperatures between 17 and 20 °C, and oxygen concentration between 8.5 and 12 mg l-1. Perch living in eutrophic (total phosphorus > 40 µl l-1), colder (maximum temperature 16 °C) and low-oxygenated (4-10 mg l-1) waters were not infected.

This study aimed at determining the prevalence of *Diphyllobothrium* sp. in perch of the Lake

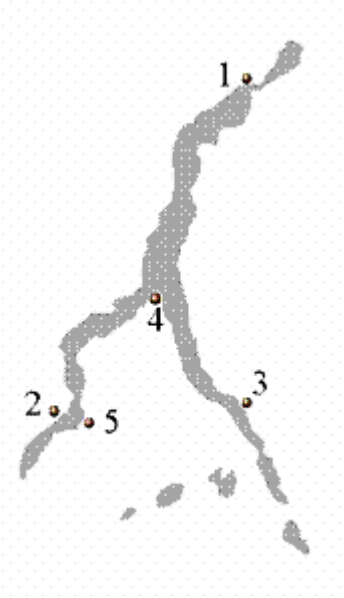

Figure 1. Sampling stations in Lake Lario, Northern Italy. 1: Domaso; 2: Carate Urio; 3: Mandello del Lario; 4: Bellagio; 5: Faggeto Lario.

Lario from 2005 to 2007, and analyzing its occurrence in its three branches (Alto Lago, western branch and eastern branch) in relation to abiotic factors. The identity of the plerocercoid larvae was confirmed using molecular methods.

Materials and methods

Prevalence

Samplings of perch (*Perca fluviatilis*) in Lake Lario were carried out from October 2005 to September 2007 in 5 stations located in the Alto Lago (Domaso), the western branch (Carate Urio, Faggeto Lario), the eastern branch (Mandello Lario) and on their confluence (Bellagio) (Figure 1). About 50 individuals (i.e. 100 fillets) were sampled for each station. Fish was provided by local authorized fishermen and was caught using gill nets. The presence of plerocercoid larvae of *Diphyllobothrium* sp. was checked by visual inspection, candling fillets by a transilluminator during the filleting activities in the field. Plerocercoids collected were fixed in 70% ethanol. Prevelances and infection intensities were calculated according to Bush et al. (1997).

All isolated plercocercoid larvae were identified morphologically on the basis of scolex and cuticle characters as described by Andersen et al. (1987) and Andersen & Gibson (1989). To confirm their identification, 3 specimens from different branches (Mandello Lario, Carate Urio and Domaso) were analyzed using molecular techniques. DNA from whole larvae was extracted using the QIAamp DNA Minikit (QIAGEN, 2003) and tested by polymerase chain reaction with specific primers for cytochrome c oxidase subunit 1 (Cox-1) gene amplification (Nakao et al., 2007), using the Taq PCR Master Mix Kit (QIAGEN, 2002). After control by agarose gel electrophoresis with 1% gel containing ethidium bromide, PCR products were purified (NucleoSpin® Extract II kit, Macherey-Nagel, Oensingen, Switzerland) and quantified by spectrophotometry (ND-100 Spectrophotometer; NanoDrop Technologies Inc., Wilmington, USA). The sequencing reaction was performed with the BigDye Terminator Cycle Sequencing Ready reaction kit (Applied Biosystems, Rotkreuz, Switzerland), according to the manufacturer's recommendations. The sequencing products were purified by filtration (MF™ membrane filters 0.025 µm; Millipore AG, Zug, Switzerland) and loaded in an automatic sequencer (ABI PRISM® 310 Genetic Analyzer; Perkin-Elmer, Rodgau-Jügesheim, Switzerland). Electropherograms were corrected with BioNumerics (Applied Maths, St-Martens-Latem, Belgium). Sequences were aligned with Cox-1 reference sequences of *D. latum* (accession numbers: AY972071 and FM209181); *D. dendriticum* (DQ768193), *D. nihonkaiense* (AB015755 and DQ768190); *D. ditremum* (DQ768195); and *Schistocephalus solidus* as outgroup (AF247839) available in GenBank. The phylogenetic tree was constructed using the Neighbor-Joining method (Kimura-2 parameters) and tested by bootstrap for 1,000 replicates (MEGA version 4.0; (Tamura et al., 2007)).

Abiotic factors

Monthly values in the epilimnion of water temperature (0-20 m), oxygen concentration (0-20 m) and total phosphorus (0-25 m), measured in 2005 and 2006 in Abbadia Lariana (eastern branch), Dervio (Alto Lago) and Como (western branch) (ARPA Lombardia, Dip. Provinciale Lecco), were analyzed graphically

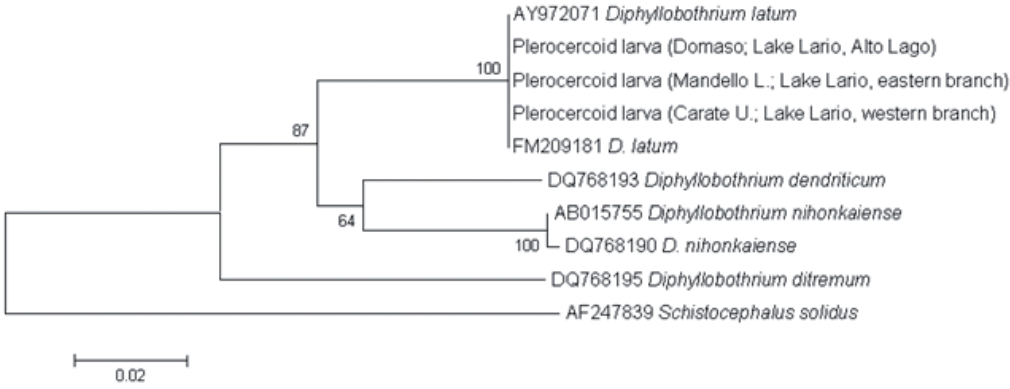

Figure 2. Phylogenetic tree of partial cox-1 gene sequences (393 bp); Neighbor-Joining method, Kimura-2 parameters. Bootstrap values for 1000 replicates shown on nodes.

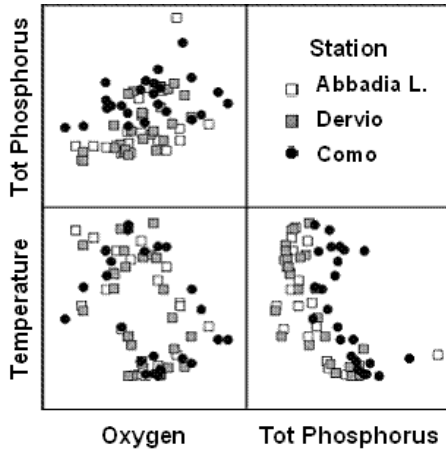

Figure 3. Scatterplot matrix based on monthly values of temperature (°C), dissolved oxygen (mg l-1) and total phosphorus (µl l-1) measured in the epilimnion for the years 2005 and 2006, in the three branches of Lake Lario. Abbadia L.: eastern branch; Dervio: Alto Lago; Como: western branch.

using scatterplot matrices prepared with SPSS 14.0 (SPSS Inc., Chicago, IL, USA).

Results

The mean prevalence of the broad tapeworm in perch of Lake Lario was 30% (n=609). Prevalence rates in the three branches were 18.7% (western; n=257), 39.2% (eastern; n=102) and 46.5% (Alto Lago; n=142) and 26.8% in the confluence (n=108). Infection intensity values ranged between 1,00 and 1,28 parasites/fish. The mean infection intensity was 1.25 parasites/fish. Molecular analysis of the plerocercoids showed 100% identity with *D. latum* reference sequences AY972071 (plerocercoid isolated from perch of Lake Léman) and FM209181 (plerocercoid isolated from perch of Lake Maggiore; Figure 2).

Scatterplot display of abiotic factors allowed to differentiate different patterns characterizing the profile of temperature to phosphorus and the profile of phosphorus to oxygen, which distinguished the western branch (Como) from the others (Abbadia L. and Dervio; Figure 3). The levels of total phosphorus in the western branch were generally between 20 and 30 μg l-1, with a mean of 23.9 μg l-1. In both the eastern branch and in the Alto Lago it was usually between 10 and 25 μg l-1, with means of 16.2 and 15.9 μg l-1, respectively.

Water temperatures and oxygenation trends were similar in all stations. Values of oxygen ranged from 8 to 14 mg l-1 (except from 5 measures), with a mean of 10.6 mg l-1 in Como (western branch), 10.4 mg l-1 in Dervio (Alto Lago) and 10.3 mg l-1 in Abbadia L. (eastern branch). Temperatures ranged from 6.2 to 21.5 °C, with a mean of 13.1 °C in Como (western branch), 12.9 °C in Dervio (Alto Lago) and 13 °C in Abbadia L. (eastern branch).

Discussion and conclusions

This study has shown that *D. latum* infection in perch is widespread in the Lake Lario. These findings confirm the reports from other Italian and Swiss lakes. The mean prevalence (almost a third of the individuals infected) in this lake is nevertheless markedly higher if compared to other Insubrian basins. Perch is among the most popular local fish consumed in this area, but other species, including imported salmonids, have been linked to the transmission of diphyllobothriosis to humans (Wicht et al., 2007; Wicht et al., 2008). Anamnestic data of some human patient included a frequent consumption of raw whitefish (*Coregonus lavaretus*), never reported as intermediate host of *D. latum.*

Man seems to be the most suitable host acting as reservoir of *D. latum* in the sub-alpine territory. Even if cats, dogs and other ichthyophagous mammals have already been mentioned as possible definitive hosts of the parasite, it could be useful to investigate the role of rats and other micromammals in the maintenance of *D. latum* life cycle.

Our results emphasize the importance of preventing infection by an effective education about the right way of preparing fish dishes. Adequate cooking (minimum 55 °C for at least 5 minutes) or deep freezing before consumption of raw fish (minimum -10°C for at least 8-72 hours, depending on the thickness of the fillets) kill plerocercoid larvae of *Diphyllobothrium* spp. (Feachem et al., 1983; Dick et al., 2001).

Abiotic factors measured in the epilimnion of the three branches of Lake Lario showed two main patterns, especially concerning their eutrophic degree. In the western branch, where the prevalence was markedly lower (18.7%) compared to the others, monthly rates of total phosphorus were consistently higher than those of both the eastern branch and the Alto Lago (39.2% and 46.5% prevalence, respectively). These data indicate that the concentration of total phosphorus could influence the occurence of *D. latum*, which seems to be higher in basins with a low trophic degree. The prevalence was nevertheless high in all sampled stations; this could be caused by well oxygenated and warm waters in the course of all year. Water temperatures of 16-20 °C and dissolved oxygen of 8-12 mg l-1 represent favourable conditions for *Diphyllobothrium* eggs embryoning and hatching (von Bonsdorff, 1977; Bylund, 2003). In Lake Lario, from June to November 2005 and 2006, water temperatures in all stations ranged from 15 to 21 °C. Oxygen concentration was between 8-14 mg l-1 during most months. These conditions seem to be ideal for the maintenance of the life cycle of *D. latum* and could explain its presence in Lake Lario. Our results confirm the data shown in previous studies (Molzen, 2005; Wicht et al., 2009).

A major limitation of our study is the sampling methodology, in particular concerning its timing. In the future, it would be interesting to sample perch in the three branches of Lake Lario at monthly intervals, to determine seasonal percentages of infection, and to verify their relationship with the respective values of water temperature, oxygenation and trophic degree.

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References

Andersen K, Ching HL & Vik R, (1987). A review of freshwater species of *Diphyllobothrium* with redescriptions and the distribution of *D. dendriticum* (Nitzsch, 1824) and *D. ditremum* (Creplin, 1825) from North America. *Canadian Journal of Zoology* **65**, 2216-2228.

Andersen KI & Gibson DI, (1989). A key to three species of larval *Diphyllobothrium* Cobbold, 1858 (Cestoda Pseudophyllidea) occurring in European and North America freshwater fish. *Systematic Parasitology* **13**, 3-9.

Bonini P, Montorfani S, Peduzzi R & Renon P, (1998). Situazione della plerocercosi nei laghi insubrici italo-svizzeri. *Obiettivi & documenti veterinari* **4**, 65-71.

Borroni L & Grimaldi E, (1973). Frequenza dell'infestione muscolare da larve plerocercoidi di *Diphyllobothrium latum* (Cestoda: Pseudophyllidaea) nel pesce persico (*Perca fluviatilis*) dei laghi italiani (anni 1968-1970). *Rivista di Parassitologia* **34** (1), 45-54.

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

Bush AO, Lafferty KD, Lotz JM & Shostak AW, (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. J*ournal of Parasitology* **83**, 575-583.

Bylund BG, (2003). 17 *Diphyllobothrium latum*. In: H. Akuffo, E. Linder, I. Ljungström and M. Wahlgren (Ed.). Parasites of the colder climates. Taylor and Francis, London & New York, pp 169-176.

Chiodera A, Ciammarughi R, Scalzini A, Badinelli MG & Sueri L, (1987). Botriocefalosi umana sul Lago d'Iseo: descrizione di 8 casi emersi in periodo breve ed in area limitata. *Rivista Italiana di Piscicoltura e Ittiopatologia* **22**, 34–37.

Dick TA, Nelson PA & Choudhury A, (2001). Diphyllobothriasis: update on human cases, foci, patterns and sources of human infections and future considerations. *Southeast Asian Journal of Tropical Medicine and Public Health* **32**, 59-76.

Dupouy-Camet J & Peduzzi R, (2004). Current situation of human diphyllobothriasis in Europe. *Euro Surveillance* **9**, 31-35.

Feachem RG, Bradley DJ, Garelick H & Mara DD, (1983). Sanitation and disease health aspects of excreta and wastewater management. In "World Bank Studies in Water Supply and Sanitation", **3**, (J.W.A. Sons, Ed.) 407-411. New York.

Magatelli PA, (1987). Alcuni casi di botriocefalosi humana emersi in breve periodo ed in area limitata. Specializzazione in Patologia Generale, 1987. Università di Pavia.

Molzen B, (2005). Die Auswirkung des Befalls mit Plerocercoiden des Hechtbandwurms (*Triaenophorus nodulosus* (P) auf den Flussbarsch (*Perca fluviatilis* L.) im Bodensee-Obersee. PhD Thesis. Institut für Zoologie, Fischereibiologie und Fischkrankenheiten. Ludwig-Maximilians-Universität München.

Morishita K, Komiya Y & Matsubayashi H , (1973). Progress of Medical Parasitology in Japan. Ed. *Meguro Parasitological Museum, Tokyo, Japan*. **5**, 129-144.

Nakao M, Abmed D, Yamasaki H & Ito A, (2007). Mitochondrial genomes of the human broad tapeworms *Diphyllobothrium latum* and *Diphyllobothrium nihonkaiense* (Cestoda: Diphyllobothriidae). *Parasitology Research* **101**, 233–236.

Parona E, (1887). Intorno la genesi del *Bothriocephalus latus* (Bremse) e la sua frequenza in Lombardia. *Archivio per le Scienze Mediche* **11**, 41-95.

Peduzzi R, (1990). Résurgence de la bothriocéphalose (parasitose à *Diphyllobothrium latum*) dans la région du lac Majeur. *Médecine et Maladies Infectieuses* **20**, 493-497.

Peduzzi R & Boucher-Rodoni R, (2001). Resurgence of human bothriocephalosis (*Diphyllobothrium latum*) in the subalpine lake region. *Journal of Limnology* **60**, 41-44.

QIAGEN, (2002).Taq PCR Handbook, pp. 38; 18-19.

QIAGEN, (2003).QIAamp DNA Mini Kit Tissue Protocol, pp. 51; 33-36.

Scolari C, (1955). Ricerche sulla frequenza della plerocercosi da *Diphyllobothrium latum* nei pesci dei laghi dell'Italia settentrionale e sulla infestione nelle diverse specie ittiche recettive. *La Clinica Veterinaria* **78**, 210-214.

Tamura K, Dudley J, Nei M & Kumar S ,(2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Molecular Biology and Evolution* **24**, 1596.

Terramocci R, Pagani L, Brunati P, Gatti S, Bernuzzi AM & Scaglia M, (2001). Reappearance of Human Diphyllobothriasis in a Limited Area of Lake Como, Italy. *Infection* **29**, 93-95.

von Bonsdorff B, (1977). *Diphyllobothriasis* in man. New York: Academic Press.

Wicht B, de Marval F & Peduzzi R, (2007). *Diphyllobothrium nihonkaiense* (Yamane et al., 1986) in Switzerland: First molecular evidence and case reports. *Parasitology International* **56**, 195-199.

Wicht B, de Marval F, Gottstein B & Peduzzi R, (2008). Imported diphyllobothriasis in Switzerland: molecular evidence of *Diphyllobothrium dendriticum* (Nitsch, 1824). *Parasitology Research* **102**, 201-204.

Wicht B, Limoni C, Peduzzi R & Petrini O, (2009). *Diphyllobothrium latum* (Cestoda: Diphyllobothriidea) in perch (*Perca fluviatilis*) of three sub-alpine lakes: influence of biotic and abiotic factors on infection rates. *Journal of Limnology* (accepted).

Woo PTK, (1996). Fish diseases and disorders. Volume 1. Ed. Cab International, Wallingford, UK. 808.