Scientific on the parasites of fish

Pasteur Institute of Lille

Amphitheater of the Biology Institute of Lille (IBL)

Monday, the 26th of Mai 2014

9h30	Welcome of the participants
10h00 – 10h40	Diversity of the parasites of fish and the risk for health The main objectives of Fish-Parasites network (ANR-10-ALIA-04) *Cécile-Marie Aliouat-Denis (BDPEE – CIIL, Lille)*
10h50 – 11h30	New insights into genetic, ecological and epidemiological aspects of anisakid nematodes - *Simonetta Mattiucci (Université La Sapienza, Rome, Italie)*
11h40 – 12h00	Sampling strategy and parasite prevalence in fish collected during research cruises - *Véronique Verrez-Bagnis (Ifremer, Nantes)*
12h10 – 12h30	Molecular identification of *Anisakidae* isolated from fish. *Yuwalee Seesao (BDPEE - CIIL Lille - ANSES Boulogne s/mer)*

Pause méridienne 12h30 – 14h00

14h00 – 14h20	*Cryptosporidium* in fish - *Gabriela Certad (BDPEE – CIIL, Lille)*
14h30 – 14h50	Diphyllobothriosis in 2014. *Jean Dupouy-Camet (Paris Descartes University, Cochin hospital, Paris)*
15h00 – 15h20	Molecular identification of *Diphyllobothrium* spp. *Hélène Yéra (Paris Descartes University, Cochin hospital, Paris)*
15h30 – 18h00	Discussion between the members of the Fish-Parasites consortium

Diversity of fish parasites and risk for health

Objectives of the Fish-Parasites network (ANR-10-ALIA-04)

Cécile-Marie ALIOUAT-DENIS and the members of the Fish-Parasites consortium.

Biology and Diversity of Emerging Eukaryotic Pathogens (BDEEP) – Centre of Immunity and Infection of Lille (CIIL) – Lille Nord of France University.

Fish carry a wide range of taxonomically diversified parasites with economical or public health impact. As an introduction to this scientific day focusing on the parasites of fish, we will give a brief overview of the taxonomical position of these different groups of fish parasites and will review a few of them namely *Cryptosporidium*, Microsporidia and Myxozoa (or Myxosporidia). As the presence of *Cryptosporidium* spp. in fish will be described in details later during the day, the impact of these coccidian parasites in shellfish is discussed here. In the last 15 years, many studies have reported the presence of either *C. parvum* or *C. hominis* in edible bivalve molluscs worldwide. But only few of them have investigated the capacity of the oocysts isolated from shellfish to elicit the cryptosporidiosis in a mammalian model. The question of whether the *Cryptosporidium* organisms are accumulating passively in these filtrating molluscs or enter actively in the shellfish hosts as an obligatory step of their life cycle, is still open.

Microsporidia parasites belong to the clad of Eumycota and some of them can be pathogen to human beings such as *Enterocytozoon* spp. or *Encephalitozoon* spp. Some Microsporidia organisms can infect fish. While they can lead to fish product alteration by forming macoscopical xenomas along the fish backbone (i.e. *Spraguea* spp. in the fish *Lophius piscatorius*), they do not seem to lead to any symptoms in fish nor are there any data of pathogenicity to human beings.

Myxosporidia (or Myxozoa) are metazoan parasites thought to be taxonomically closed to Cnidaria. Among many species infecting fish, *Myxobolus cerebralis* is the most notorious as it is the causing agent of whirling disease that killed a big proportion of salmonids worldwide with high economical loss. Other species such as *Kudoa* spp. can form white ellipsoid cysts modifying the texture of the fish flesh. Spores of Myxozoa have been reported in human stools. The link between the occurrence of myxozoan parasites and clinical symptoms in humans is discussed.

To conclude, a broad overview of the main objectives of the Fish-Parasites network is presented as well as some practical advices to avoid parasite infection while eating fish.

Scientific day on the parasites of fish

New insights into genetic, ecological and epidemiological aspects of anisakid nematodes

Simonetta MATTIUCCI

Université La Sapienza, Rome, Italie

No abstract available

Sampling strategy and parasite prevalence in fish collected during research cruises.

Véronique VERREZ-BAGNIS* and the members of the Fish-Parasites consortium.
**Ifremer, BRM-EMB, Nantes, France.*

Some parasites of marine fish, such as nematodes (*Anisakidae*), are zoonotic pathogens that have an impact on public health. Given the increasing consumption of seafood in France and especially of raw fish, the Fish-Parasites action aims at: (i) identifying parasites in fish most commonly consumed, (ii) exploring the potentially structuring role of host species, geography and other factors on parasite dsitribution, (iii) providing technical strategies to improve the detection of the parasite in fish fillets, (iv) creating a platform to identify parasites in fish and (v) providing continuing education sessions to professionals.

Fifteen fish species were selected based on a risk analysis that took into account the French consumption, the level of consumer exposure and the level of infestation by anisakid (literature data). Fish were sampled either on boats during research campaigns or inland (fish markets or fishing companies), and dissected to collect the parasites. Nematodes were identified to species using molecular identification. All data on sampling, fish individuals, and parasites were collected in a database PARAFISH developed for the project.

Here, only the data on the parasite infestation level of fish sampled during research campaigns are described. Parasite prevalence is strongly dependant on the fish species and on geographical areas (Mediterranean sea, Atlantic ocean and Channel and North sea). In total, 505 fish over the 879 fish sampled during the scientific campaigns harboured at least 1 macroscopical parasite (57.5% of prevalence). Corporal cavity represented 40% of the fish location infested by nematodes, whereas liver and fillet represented 25% and 21% respectively.

Given the fact that Anisakids from 147 fish have not been identified to the species level, the data showed that *Anisakis simplex* is found, at least, in 455 fish “organs”, and *Anisakis pegreffii* and *Hysterothylacium aduncum* are found, at least, in 96 and 91 fish “organs”, respectively. Only two species, *A. pegreffii* and *H. aduncum*, were found in fish sampled in Mediterranean sea, whereas *A. simplex*, *Contracaecum sp* and *H. aduncum* were found in fish sampled in Channel and North sea. Fish sampled in Bay of Biscay and Celtic sea are more infested than fish sampled in other areas, and the nematode species found are mainly *A. simplex*, *A. pegreffii*, *H. aduncum*, *Contracaecum sp*, *Pseudoterranova decipiens* and *P. krabbei*.

The data on the identification of the nematode species and their prevalence will be further analysed, both on fish sampled inland and during Ifremer research cruises, to determine the potential structuring role of some factors on the distribution of nematodes.

Scientific day on the parasites of fish

Molecular identification of *Anisakidae* isolated from fish.

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Numerous parasites frequently infest edible fish worldwide. Among them, the *Anisakis*, *Contracaecum* and *Pseudoterranova* genera, that belong to the *Anisakidae* familly, are nematodes whose larvae are found in numerous fish and cepholopod species. Ingestion of these larvae may induce digestives and/or allergic pathologies in humans. In France, the consumption of raw (or slightly cooked) fish products, is rising. Consequently, we aimed at better defining the impact of fish parasites on the consumer health as well as efficient prevention strategies to reduce the risk for consumers of fish products.

As only few data are available on the Anisakid distribution in fish from France, on of the objectives of the Fish-Parasites action (ANR-10-ALIA-004) is to collect and identify *Anisakidae* nematodes. The fish species sampling scheme has been established on the basis of a risk ranking analysis taking into account the consumption data of the fish in France and the prevalence data of *Anisakidae* available in the literature. The parasites are identified by amplifying and sequencing a fragment of the locus *COX2*. Nematodes from 15 species (1675 fish) sampled in the North-East Atlantic ocean, the bay of Biscay, the Ireland sea, the Mediterranean sea, the Channel and the North sea were isolated from fillets, corporal cavity, liver and digestive tract. If an organ contains more than 11 nematodes, genomic DNA is extracted from the pool of nematodes and a newly set-up PCR allowed to produce a library of sequences at the *COX2* locus which is then sequenced at a high throughput (HTS) on the PGM Ion Torrent® platform (Life Technologies). If only few nematodes are present per organ, the classical Sanger sequencing at the same locus is performed. An automated pipeline of analysis is accessible via the Galaxy interface. The DNA sequences are thus compared to a library of reference sequences constituted from available sequences for the needs of the study. *In fine*, the molecular identification will allow to determine the relative frequencies of the various nematodes taxa occurring in one given organ.

Preliminary results of the global prevalence of the nematodes infecting the 1675 sampled fish are as follows: 46,75% of the fish examined are free of nematodes, 32,98% of the fish are infested by nematodes in the viscera, 2,58% carry nematodes only in the fillets and 17,68% carry nematodes both in the fillets and in the viscera. The first HTS run identified 8305 nematodes isolated from de 92 organs (fillet, liver corporal cavity) of 11 fish species. The genus *Anisakis* represented 93,8% of the nematodes. Nonetheless, 6,2% of the sequences remained non-identified and may correspond to new sub-types or species.

These frequency data will allow to: (i) evaluate the distribution of the different parasite species in fish organs and (ii) analyse the impact of some variables, such as the fish species, the fish size, the fishing area and season on the *Anisakidae* distribution in fish in order to set up prevention measures.

***Cryptosporidium* in fish.**

Gabriela Certad and the member of the Fish-Parasites consortium.

Biology and Diversity of Emerging Eukaryotic Pathogens (BDEEP) – Centre of Immunity and Infection of Lille (CIIL) – Lille Nord of France University.

Introduction. The *Cryptosporidium* organisms are intracellular Apicomplexa protists. The genus *Cryptosporidium* consists of species that infect the intestine of numerous vertebrates, including human. The *Cryptosporidium* species are the causing agents of a cosmopolitan zoonosis: the cryptosporidiosis, an emerging opportunistic disease with considerable impact in immunosuppressed patients, in whom cryptosporidiosis may become chronic and lethal. The transmission route of cryptosporidiosis is oro-faecal by ingestion of oocysts occurring in water or food contaminated by stools or by contact with an infected subject. The infection may also develop in immunocompetent subjects, sporadically or as epidemics; but in that case, the clinical symptoms (diarrhoea) are spontaneously resolving, generally without complications. In fish, two species, *Cryptosporidium molnari* and *Cryptosporidium scophthalmi* have already been described. Recently, in Australia, novel species/genotypes have been identified in fish thanks to molecular tools. The main objective of this study is to evaluate the prevalence of *Cryptosporidium* spp. in fish products from France including fresh water and marine fish.

Materials and methods. Overall, 42 fish bought in the Thonon-les-Bains (Geneva Lake, France) fishing harbour, in November 2011 (autumn) and April 2013 (spring) as well as 1470 marine fish (collected between 2011 and 2013) were analysed to detect the presence of *Cryptosporidium* spp. by nested-PCR from gastric and intestinal scrapings. The nested-PCR-targeted locus is a fragment of the gene encoding the 18S rRNA. The amplified products are sequenced and compared to available sequences of public databases (NCBI). The tissues of gastric and intestinal mucosa were sampled for histological analyses.

Results. The *Cryptosporidium* spp. have been detected in 15 fish samples collected from the Geneva lake, which represents a prevalence of 33,6% distributed as follows: 86% (13) of *C. parvum* positive samples, 7% (1) of *C. molnari* positive samples and 7% (1) of *C. molnari* / *C. parvum* mixed infection. In marine fish, *Cryptosporidium* spp. have been detected in 28 samples, which represents a 2% prevalence distributed as follows: 25% (7) and 75% (21) of *C. parvum*- and *C. molnari*-positive samples, respectively. In total, 5 species of fresh water fish and 11 species of marine fish were identified as new hosts for *Cryptosporidium*. The histological examination of tissues from *C. parvum* infected fish revealed the presence of several progressive parasite stages at the apical border of both gastric and intestinal epithelia.

Conclusions. In our study, two *Cryptosporidium* species were detected in fish: (i) *C. molnari*, a species already described in marine fish but identified here for the first time in fresh water fish and (ii) *C. parvum*, a species already described in fish but mainly considered as a mammal-infecting species. Our histological analyses demonstrated the presence of *C. parvum* in the epithelial cells of the digestive tract. These data speak in favour of a *C. parvum* infection in fish rather than a passive carriage. The occurrence of a *C. parvum* sustained life cycle in fish would constitute a marker of faecal pollution of the environment. *Cryptosporidium* is more frequently detected in fresh water rather than in marine fish. This could be explained by several factors such as lake contamination by sewage or livestock farming nearby. These results may impact on the public health, as *C. parvum* is a zoonotic species whose dispersion is facilitated by the aquatic habitat of the host.

Prevalence of *Diphyllobothrium latum* infestation in different fish species from three French sub-alpine lakes (2011-13) and trends in the evolution of the human parasitosis.

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Diphyllobothrium latum, the fish tapeworm, is the biggest tapeworm in humans. It causes a parasitic infection called diphyllobothriosis which is acquired by eating raw fish. Surveys performed 10 years ago indicated that human diphyllobothriosis was emerging or re-emerging in some European countries (in the Italian and French speaking sub-Alpine regions) and particularly around the lake of Geneva (Dupouy-Camet & Peduzzi, Eurosurveillance, 2004;9:31-5). In the Leman Lake, previous works of our team (Nicoulaud et al., Parasite, 2005, 12, 362–364) reported a 4 to 10 % prevalence of plerocercoids in perch fillets (*Perca fluviatilis*).

The present work aimed to evaluate: the evolution of the prevalence of *D. latum* in perch fillets, the prevalence of this parasite in different fish species and the incidence of the human parasitosis. Perch fillets and fish were bought from professional fishermen of the Leman Lake or obtained after scientific fisheries (J. Guillard, INRA) in the Annecy and Bourget Lakes. All perch fillets (n = 1013) were examined by scrapping the fillets with a kitchen knife or by candling. Most obtained plerocercoids were identified by PCR targeting the COI or NADH genes as previously described (Yera et al., Parasite. 2008;15:402-7).

A global prevalence of 2.2% was found ranging from 0% to 23% according to geographical location or weight of the fillet. A positive correlation was found between the weight of the perch fillet and the prevalence of plerocercoids. Dissection of 138 fish (ranging from 14g to 3000g) from several species coming from Annecy, Bourget and Leman Lakes were performed. Fish parasitized with *D. latum* were only observed in the Leman Lake where plerocercoids were found in the muscles or in the general cavity of 7/7 pikes (*Esox lucius*), 7/24 perches (*Perca fluviatilis*) and of 2/7 burbots (*Lotta lotta*). No pleroceroids were found in any of the 8 chars (*Salvelinus alpinus*) and of the 13 white fish (*Coregonus lavaretus*). No *D. latum* plerocercoid was found in any of the 51 perches, 9 pikes and 5 burbots from the Bourget and Annecy lakes. The highest numbers of plerocercoids were found in pike (up to 18 in a single fish). Pike and burbot, always consumed well done by the consumers, do not represent an important public health risk. This is not the case for perch which can be consumed raw (*carpaccio di persico* or *filets de perche marinés*).

We have observed a decrease of the prevalence of parasitized perches during the 3 years of our study and compared to the prevalence observed ten years ago. This decrease was also correlated with a decrease of the incidence of human cases in the region as only 6 cases were reported during the past 3 years compared to the 44 cases (7.3 case/year reported in the same region between 2002-2007 (Wicht PhD, University of Geneva., 2009). Information to consumers and professionals may be responsible for this encouraging evolution.

Molecular identification of *Diphyllobothrium* spp.

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We have confirmed the importance of the molecular diagnostic of *Diphyllobothrium* species allowing the diagnosis of exotic diphyllobothriosis or the detection of genera close to *Diphyllobothrium*, that could be pathogenic to humans (Yera *et al*., Parasitology International, 2013, 62:268-71).

In this work, we aimed at developing and validating molecular tools necessary for the identification of *D. latum* and for the study of its genetic diversity. As numerous larvae are to be identified, we developed a method for *D. latum* rapid identification such as a specific real-time PCR. Our technical choice is based on its sensitivity (low quantity of inititial material required), its rapidity (to save time) and its specificity. A real-time PCR specific to the *Diphyllobothrium* genus was thus designed using the SYBER® Green intercalating agent and the analysis of fusion curves. The fusion temperatures were too closed for *D. latum* and the other species and did not allow the identification of *D. latum* isolates. Next, we designed a TaqMan® real-time PCR with a probe that we had expected to be specific for *D. latum*. Unfortunately, this attempt also led to the detection of other *Diphyllobothrium* species. Finally, a TaqMan® real-time PCR with specific primers and probe of *D. latum* was designed. Only the *D. latum* isolates were detected. More isolates should be tested to confirm these last results.

We have detected a polymorphism between the different isolates of *D. latum*. It is characterised by a variation of the number of repeats (4 to 8) of a 36 bp-long sequence in the mitochondrial genome of *D. latum*. Five different haplotypes were noted. The low number of tested samples does not enable to draw any conclusions about the epidemiology or the geographical origin of the isolates (Haidar *et al*., unpublished data).