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REVIEW

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Biology, Epidemiology, Clinical Features, Diagnosis, and Treatment of Selected Fish-borne Parasitic Zoonoses

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Fish-borne parasites have been part of the global landscape of food-borne zoonotic diseases for many decades and are often endemic in certain regions of the world. The past 20 years or so have seen the expansion of the range of fish-borne parasitic zoonoses to new geographic regions leading to a substantial public health burden. In this article, we summarize current knowledge about the biology, epidemiology, clinical characteristics, diagnosis, treatment and control of selected fish-borne helminthic diseases caused by parasitic roundworm (*Anisakis*), tapeworm (*Dibothriocephalus*), and fluke (*Metagonimus*). Humans acquire infection via consumption of raw or improperly cooked fish or fish products. The burden from these diseases is caused by morbidity rather than mortality. Infected patients may present with mild to severe gastrointestinal (eg, abdominal pain, diarrhea, and indigestion) or allergic manifestations. Patients are often admitted to the hospital or clinic with acute symptoms and no prior health problems and no travel history. Diagnosis is often established based on the detection of the diagnostic parasite stages (eg, eggs or tapeworm segments) in the patient's feces. Sometimes imaging is required to exclude other causes and avoid unnecessary surgery. *Dibothriocephalus* and *Metagonimus* are mainly treated with praziquantel. Extraction of adult *Dibothriocephalus* or *Anisakis* larvae from the bowel ensures complete elimination of the parasites and prevents a relapse of infection. The development and implementation of more efficient food safety and public health strategies to reduce the burden of zoonotic diseases attributable to fish-borne parasites is highly desirable.

INTRODUCTION

Among the etiological agents of emerging zoonotic diseases are several parasitic helminths (worms) that naturally reside in fish and belong to diverse taxonomic groups, including roundworms, tapeworms, and flukes. In recent years, fish-borne parasitic helminthiasis has emerged as a major food safety concern which can impose significant public health and economic impacts [1]. By the start of the new millennium, fish-borne zoonotic trematodes accounted for more than an estimated 18 million infections [2]. The available evidence suggests that

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Abbreviations: GI, gastrointestinal; L3, third-stage larva

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marine and freshwater fish, or fish products, particularly those originating from wild-caught fish, are the main source of transmission of fish-borne parasitic infections [3,4].

Although fish-borne parasites are reported worldwide, their prevalence is disproportionately higher in Asian countries, where fish is the major source of protein [5] and where local communities favor the consumption of raw fish. In certain Eastern Asian countries, such as Korea, raw fish restaurants are common and raw fish consumption festivals are even supported by local authorities [6]. Youth are more likely to participate in these events which increases their chances of being infected by fish-borne parasites [6]. In the last few decades some Western countries have gone through significant changes in dietary and cultural habits, such as the growing popularity of eating raw or minimally cooked fish, especially sashimi, sushi, ceviche, and carpaccio, or fish tartare, which increases the risk of exposure to the infective stages of various fish-borne parasites [7,8].

Effective control of fish-borne parasitic infections has been challenging due to various factors, such as international tourism and global trade of fresh fish on ice [9]. Changes in fish handling and keeping fish intact, without evisceration, after being caught put fish consumers at risk of acquiring parasite infection [10]. Also, importation of exotic fish, travel to countries where fish-borne parasites are prevalent, and the growing interest in the consumption of raw fish delicacies have introduced exotic fish-borne parasites to European countries, such as France, Switzerland, and Finland, as well as New Zealand [9,11-13]. Not surprisingly, parasites associated with fish (eg, anisakid nematodes, diphyllobothriidean tapeworms, intestinal heterophyid, and opisthorchid liver flukes) are ranked among the top food-borne parasites globally [14] and in Europe [15].

These challenges have led to a growing interest in the development of educational campaigns in some countries to inform fish consumers and professionals about the dangers of raw fish consumption [12,16,17]. However, these measures are not strictly followed by consumers and the incidence of infections by fish-borne parasites has not subsided. One major obstacle to achieve better public compliance is the fact that fish-borne diseases are not life-threatening, and thus attract little attention from both the public and health professionals [18]. One of the key components of an efficient control and prevention program is to create awareness about fish-borne diseases.

Therefore, this review focuses on notable examples of fish-borne parasitic diseases – anisakiasis, diphyllobothriasis, and metagonimiasis – caused by worms belonging to the three major taxonomic groups of parasitic helminths – nematodes, cestodes, and trematodes, respectively. We discuss current knowledge about their biology, epidemiology, clinical characteristics, diagnosis, treatment, and control.

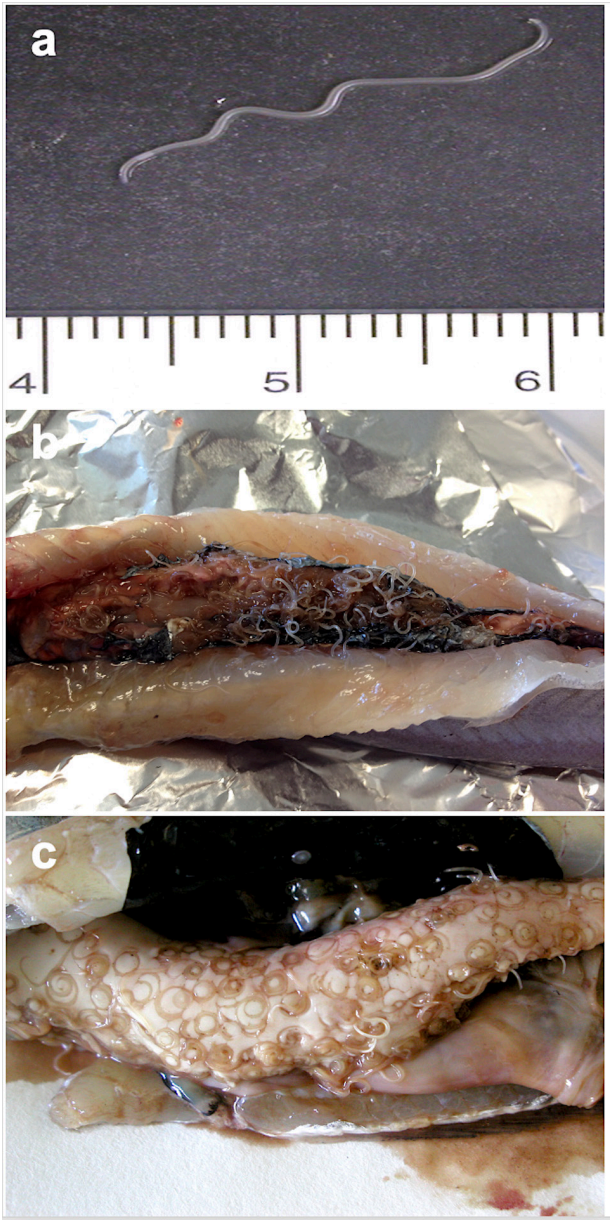


Figure 1. ***Anisakis simplex*** **in the second intermediate/paratenic host blue whiting (*Micromesistius poutassou*) fish from Spain**. (**a**) *A. simplex* third-stage larvae (L3s) isolated from *M. poutassou*. (**b**) A large number of L3s infiltrating the fish visceral organs. (**c**) The impressions (footprints) caused by L3s on the surface of the fish liver. (Photo credit: Prof. F. Javier Adroher, University of Granada, Spain)

METHODS

Literature Search Strategy

We searched PubMed, Google Scholar, SCOPUS,

and MedRxiv using the search terms "*Anisakis*," "*Diphyllobothrium*," "*Dibothriocephalus*," "*Metagonimus*," and "fish-borne" for published studies up to December 15, 2020. We also searched the references of some select articles to identify more pertinent articles. The literature search was limited to reports that have relevance to both biology and/or general medicine readership.

ANISAKIASIS

Anisakiasis, also known as anisakiosis (infection by larvae of genus *Anisakis*) or anisakidosis (infection by larvae of family Anisakidae), is associated with gastrointestinal (GI) and allergic manifestations in humans. It is caused by species of the genus *Anisakis* (Nematode: Anisakidae) [5,19,20]. *Anisakis simplex* (*sensu lato*) complex consists of *A. simplex* (*sensu stricto*), *A. pegreffii*, and *A. berlandi*. The first two *Anisakis* species are the most common causative agents of human anisakiasis. Humans acquire the infection with *A. simplex* (*s.l.*) via ingestion of infective third-stage larvae (L3s) (Figure 1a) which are found in the muscle and on the viscera (Figures 1b-c) or free in the body cavity of a number of marine fish and squid [20]. Due to the limited access to the hosts required to support the development of anisakid parasites in fish farms, *Anisakis* spp. are expected to be less prevalent in farmed than wild-caught fish [21-23]. The risk of introduction and commercialization of farmed Atlantic salmons containing viable anisakid nematodes is negligible or very low [24]. Nonetheless, *A. simplex* (*s.s.)* has been detected in farmed salmon [25] and 0.7% *A. pegreffii* infection has been reported in farmed Mediterranean sea bass [26]. Although, anisakiasis has been problematic in Far East Asian countries, especially Japan [27], the prevalence of this disease has increased in Western countries because of changes in food consumption habits.

Biology and Epidemiology

Anisakids have a complex biology (Figure 2) because their life cycle is heteroxenous and requires marine mammals and cetaceans as definitive hosts, with small crustaceans as the first intermediate hosts and squids and fish as the second intermediate hosts [28].

Anisakiasis is prevalent in Asia and Western Europe, where most of the cases are reported from Japan, and the remaining cases have been reported mainly in Germany, France, the Netherlands, and Spain. The worldwide increase in the prevalence of anisakiasis is more likely attributed to improved awareness and new advances in diagnostic methods, particularly imaging modalities. Before the advent of the gastrofiberscope, many anisakiasis cases with gastric involvement were probably unrecognized or misdiagnosed [29]. The ever-growing habit of eating raw, lightly-cooked, smoked, or marinated fish and

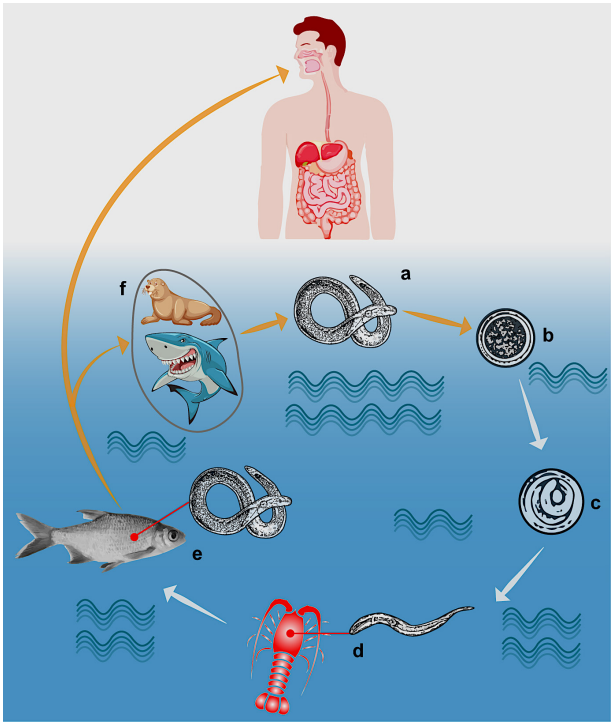


Figure 2. **Life cycle of anisakid roundworms**. (**a**) Adult anisakids live in the gastric chambers of the definitive hosts (marine mammals) and the females lay eggs, which are excreted in the feces (**b**). After maturation, embryonated eggs (**c**) hatch in the water into free-swimming larvae (**d**). These larvae are eaten by crustaceans (first intermediate hosts) where they develop inside their hemocoel. The fish and cephalopod molluscans, such as squid serve as the second intermediate hosts (**e**), eat crustaceans containing these larvae which then cross through stomach or caeca of fish and encapsulate on the viscera or free in the body cavity. All the intermediate hosts can also act as paratenic hosts, which is vital for the maintenance of infection in a given area and for facilitating the infection of the definitive hosts. (**f**) The definitive hosts are infected by eating fish or cephalopods containing L3s. Humans are accidental hosts of anisakid worms if they acquire infection via ingesting raw or minimally cooked fish or squid with L3s. Generally, the parasites do not develop further within the human gut. Adapted from the Centers for Disease Control and Prevention DPDx website (https://www.cdc.gov/parasites/anisakiasis/biology.html)

the increasing global demand for seafood may have also contributed to the risk of acquiring infection [30]. Salted or smoked herring, lomi-lomi, sushi and sashimi, ceviche, and gravlax have become popular dishes in the cuisine of many countries. The possibility of anisakid larvae occurring in specialty dishes increases if the fish used in the preparation of these dishes were not eviscerated soon after capture because L3s living on the viscera or in the body cavity may spread to the fish muscle *post mortem*.

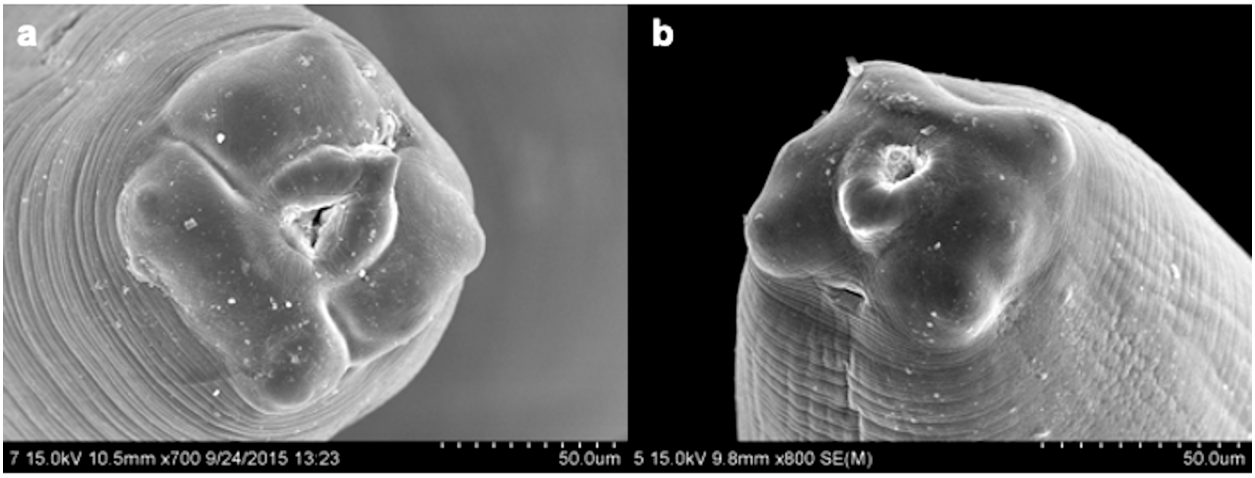


Figure 3. **Scanning electron microscope micrographs of *Anisakis* third-stage larvae**. The anterior (head) end shows the lips surrounding the mouth opening of (**a**) *A. simplex* s. s. and (**b**) *A. pegreffii*. (Photo credit: Prof. Liang Li, Hebei Normal University, China)

The improved regulatory control procedures over the unnecessary overexploitation of marine animals may have also contributed to the increasing numbers of marine mammal populations, which support the development of anisakid life cycle as definitive hosts [29-31], and thus increase the number of infected fish and the size of the parasite population.

Clinical Symptoms

Patients can present with different symptoms depending on the site of lesions caused by the infecting larvae. Asymptomatic infection occurs when the larvae stay in the GI lumen without any adverse impact on the health of the host. However, *Anisakis* larvae can invade the stomach or intestinal mucosa, or occasionally migrate to other extra-GI locations such as the throat [29,32,33]. The larvae have incipient lips, which allow them to burrow into the gut mucosa (Figure 3). Invasive infections are associated with edema and congestion, with the larvae embedded in inflammatory cell infiltrates in the stomach or intestinal mucosa [34]. Gastroallergic anisakiasis is the most common clinical form [35]. Anisakiasis can also be seen in the intestine and occasionally at ectopic sites [36]. Symptoms resulting from gastric infection seem to appear 1–8 hours post ingestion of infected fish, whereas intestinal infection often manifests after 5–7 days. Several symptoms can occur in an individual with anisakiasis, including low-grade fever and GI symptoms (eg, tenderness of the abdomen, abrupt and severe epigastric pain, nausea, dyspepsia, diarrhea, vomiting) [29]. Some individuals may exhibit intestinal obstruction, perforation, peritonitis, and bleeding.

Some patients develop allergic reactions, characterized by swelling, angioedema, urticaria, or even anaphylaxis [27,37-40]. Clinical cases of allergy together with an elevated specific antibody response to *A. simplex* were reported mainly in patients in Spain. The *A. simplex* allergens, which elicit an allergenic reaction, are relatively tolerant to freezing and heating [40], suggesting that allergic reaction may occur via ingestion of contaminated fish products processed in a manner that would deactivate the parasite. Therefore, ingestion of viable L3s in raw or insufficiently cooked fish is not the only mechanism by which *Anisakis* can cause illness; fish products may contain dead larvae [17] which, if consumed, can also provoke a hypersensitivity reaction [7,41], mediated by increased IgE sensitization [7,42]. However, more evidence indicates that exposition to viable larvae is necessary for sensitization to *Anisakis* and the development of allergic symptoms [38,43,44]. It remains to be confirmed whether initial sensitization occurs due to direct exposure to allergens derived from non-viable L3s, or a priming exposure to live parasites is necessary to cause sensitization.

Diagnosis

Clinical diagnosis of anisakiasis is generally based on examination of the presenting symptoms and patient history – particularly dietary habits [27] as anisakiasis is more likely in individuals with a recent history of consumption of raw or uncooked fish [39]. An accurate diagnosis is crucial because clinical presentation may determine the clinical management of patients. There are three clinical forms of anisakiasis, gastric, intestinal, and ectopic [45].

In gastric anisakiasis, physical examination can reveal moderate tenderness in the epigastric region, which can be misdiagnosed as a peptic ulcer. Thus, diagnosis using a more definitive method such as upper endoscopy

can accurately detect gastric anisakiasis [46]. Diagnostic imaging using upper GI endoscopy can reveal a filiform ~15 mm larva firmly adherent to inflamed and swollen mucosa, with the anterior extremity embedded in the stomach mucosa [47].

Cases of intestinal anisakiasis are not only rare, but their diagnosis has also proven challenging due to the non-specificity of symptoms; intestinal anisakiasis can be misdiagnosed as appendicitis, peritonitis, intestinal obstruction, or acute celiopathy [29]. Also, due to its anatomical location, the small intestine is often unreachable by standard endoscopic examination. Diagnosis can be confirmed by exploratory laparotomy [48]. Also, imaging modalities such as capsule endoscopy or double-balloon enteroscopy are being harnessed to support the diagnosis. However, these procedures are invasive and can be associated with complications. Computed tomography scans can reveal localized swelling and edema of the small bowel [49]. Ultrasound scans can show marked local edema and ascites [50].

Laboratory abnormalities detected in anisakiasis include mild to severe leukocytosis and elevated serum level of the inflammatory, acute-phase reactant indicator C-reactive protein, however peripheral eosinophilia is rare [45,48,50,51]. In addition to imaging, diagnosing anisakiasis can be achieved by using serological assays for the detection of anti-*Anisakis*-specific IgA/IgG, and IgE antibodies. The sensitivity of the serological assays can reach 70%-80% [52]. However, their performance can be compromised by antigenic cross-reactivity with other related roundworm species [53] and the results may take several days. Serological assays are therefore not useful in the case of invasive anisakiasis, however it is the best method for the diagnosis of *Anisakis* allergy.

Treatment

Physical removal of *Anisakis* larva adhering to the gastric wall using an endoscope is often curative without further pharmacological treatment. The *Anisakis* larva can be removed with a Roth net and this seems sufficient for resolving the clinical manifestations [51]. Conservative therapy usually improves the clinical condition and symptoms associated with acute inflammation subside within 2-3 weeks [54]. Anthelmintics, such as albendazole, can be used although they are not highly efficacious [55]. Surgical intervention is required in severe cases associated with strangulation or segmental stenosis of the intestine [49]. Patients may undergo surgical intervention if their intestinal manifestations are misdiagnosed as acute abdominal or intestinal obstructions [39,46,49,56]. Therefore, correct diagnosis of intestinal anisakiasis using diagnostic imaging is essential to avoid any unnecessary surgical intervention.

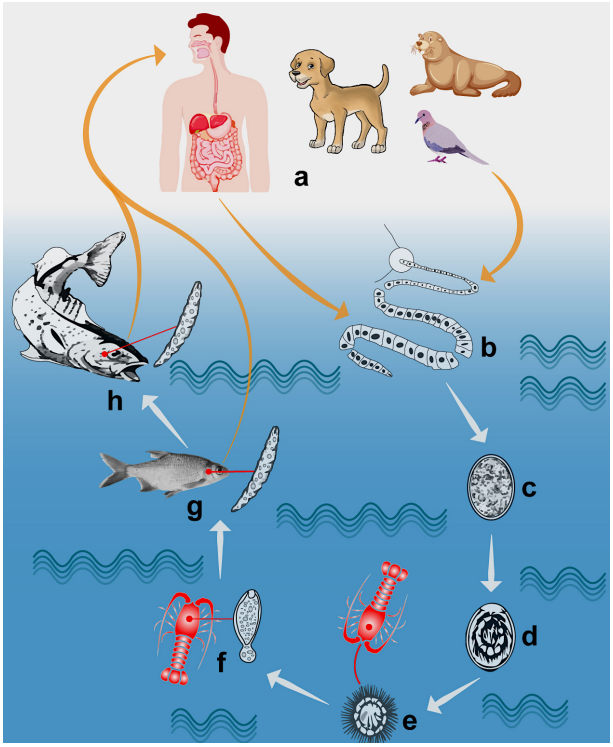


Figure 4. **Life cycle of diphyllobothriid tapeworms**. The definitive hosts (**a**), such as piscivorous birds and mammals (including humans), harbor the adult tapeworms (**b**) in their intestine, where unembryonated eggs released from tapeworms are excreted in feces (**c**). Eggs mature within approximately 3 weeks (**d**) and hatch in water, releasing ciliated larvae known as coracidium (**e**), which are ingested by a copepod crustacean water flea, such as *Cyclops* spp. (the first intermediate host) and transform into procercoid larvae in their body cavity (**f**). Various small freshwater and marine fish especially anadromous species (ie, living in both fresh and saltwater) act as secondary intermediate hosts. Following ingestion of procercoid-containing copepods, the procercoid larvae migrate to the fish musculature where they transform into plerocercoid larvae (**g**). These infected small fish can be eaten by larger predator species that serve as paratenic hosts (**h**). The definitive hosts are infected after feeding on small or larger fish containing infective plerocercoids, which are released in the intestine and attach to the intestinal lining using the bothria where they mature into adult tapeworms. Adapted from the Centers for Disease Control and Prevention DPDx website (https://www.cdc.gov/parasites/diphyllobothrium/biology.html)

DIPHYLLOBOTHRIASIS

Diphyllobothriasis (or diphyllobothriosis), a fish-borne helminthic disease caused by the broad fish tapeworms of order Diphyllobothriidea, is responsible for about 20 million human infections worldwide [57]. Recent taxonomic revision has reassigned the genus

Diphyllobotrium to other genera. For example, some of the most common species associated with freshwater fish have been integrated into the genus *Dibothriocephalus* (eg, *Diphyllobothrium latum* is now known as *Dibothriocephalus latus*), while those associated with marine fish have been integrated into the genus *Adenocephalus* (eg, *D. pacificum* is now known as *A. pacificus*). Although *D. latus* is the most prevalent species causing diphyllobothriasis, other diphyllobothriidean species can also infect humans [57,58].

Biology and Epidemiology

The diphyllobothriidean cestodes have a complex biology as illustrated in Figure 4. Similar to anisakiasis, diphyllobothriasis is caused by consumption of raw or inadequately cooked fish containing infective parasite larvae. Human infections have been usually associated with freshwater fish from Europe (Baltic countries, France, Italy, Russia, Scandinavia, and Switzerland) and North America (Pacific Northwest). However, clinical cases in Asia (Japan and South Korea) and in South America (Brazil, Chile, and Peru) have also been reported [12,59].

In Korea, the most common source of infection is the consumption of salmon, mullet, and trout [6,60]. Infection does not seem to be influenced by the gender or age of the host, but is rather determined by cultural habits [6]. A locally acquired infection with *D. nihonkaiense*, a highly prevalent fish-borne tapeworm in Japan [61], was detected in Switzerland [10]. Additionally, a case of diphyllobothriosis caused by *D. nihonkaiense* was reported in France following ingestion of Pacific salmon imported from Canada [62]. These cases show how changing eating habits can lead to an increase in illnesses related to fish-borne parasites in new geographic regions.

Clinical Symptoms

Diphyllobothriidean tapeworms infect the small intestine, however, some segments of the worm have been detected in the upper colon [6]. GI symptoms appear about 3 weeks following the consumption of infected fish. Most infected patients experience mild symptoms such as intermittent abdominal pain, abdominal distension, diarrhea, indigestion, dyspepsia, and vomiting [6]. Severe abdominal pain has been also reported [63]. Other symptoms are nonspecific, such as fever, anorexia, fatigue, myalgia [60], and even depression and anxiety. Some patients may exhibit numbness of extremities, asthenia, and vertigo [12]. Patients may spontaneously discharge tapeworm proglottids in their feces [60], which often triggers the patients to seek medical advice [60]. Colonoscopic examination can reveal the presence of motile creamy-white proglottids in the sigmoid colon. The tapeworm feeds on intestinal chyme and vitamin B12 – leading to vitamin B12 deficiency. Megaloblastic anemia is a complication of long-term infection, resulting from vitamin B12 malabsorption and a deficiency of cobalamin [64].

Diagnosis

Initial diagnosis of diphyllobothriidean infection is based on microscopic detection of eggs (~65 µm x 45 µm) in the patient's feces (Figure 5a), which can be mistaken with those of trematodes such as *Paragonimus* spp. or *Fasciola hepatica*. However, *F. hepatica* eggs are larger in size (~140 µm x 76 µm) and eggs of *Paragonimus* spp. are somewhat larger than those of *D. latus* (~100 µm x 57 µm) and much less abundant since *Paragonimus* is a pulmonary parasite and the eggs can be better detected in sputum. Adult tapeworms may live for many years, and are considered the longest human parasites as they can reach up to 20 m [6,65]. Spontaneous discharge of the entire or terminal pieces of the tapeworm strobila (a long chain of proglottids/segments) in the patient's feces often occurs in the morning. These expelled parts separated from the tapeworm strobila can be identified visually or with a stereomicroscope.

The head region in the anterior end (known as scolex) possesses two elongate grooves called "bothria," which helps the tapeworm to attach to the intestinal mucosa. Each proglottid in the strobila contains a single set of male and female genital organs (ie, hermaphroditic). The unambiguous location of genital pores in the center of the ventral surface of proglottids and the presence of a characteristic rosette-shaped uterus are confirmative of diphyllobothriidean species. *D. latus* is colloquially known as the broad tapeworm because their proglottids are broader than long. These attributes make *D. latus* morphologically distinguished from other tapeworms infecting humans, such as *Taenia* spp. which have square-shaped mature segments with laterally located genital pores and longer than broad gravid segments.

Occasionally, diagnosis may be achieved by finding and recovering motile tapeworms by gastroduodenoscopy or by colonoscopy, which helps to rule out other intestinal pathologies [6]. Most human cases are infected with a single tapeworm [6], however patients with 3 tapeworms have been reported [66]. Complete removal of the entire tapeworm, including the scolex, from the patient's intestinal tract is essential to prevent relapse of infection. However, as *D. latus* is the longest worm infecting humans it is often not expelled completely even with the use of anthelmintic drugs.

Ultrasonographic examination of the abdomen can show a hyperechoic ribbon-like structure (Figure 5b) moving freely within the intestinal tract (see video 1) [67]. Gastroduodenoscopy and colonoscopy may fail to reach to the scolex because tapeworms attach with their scolex

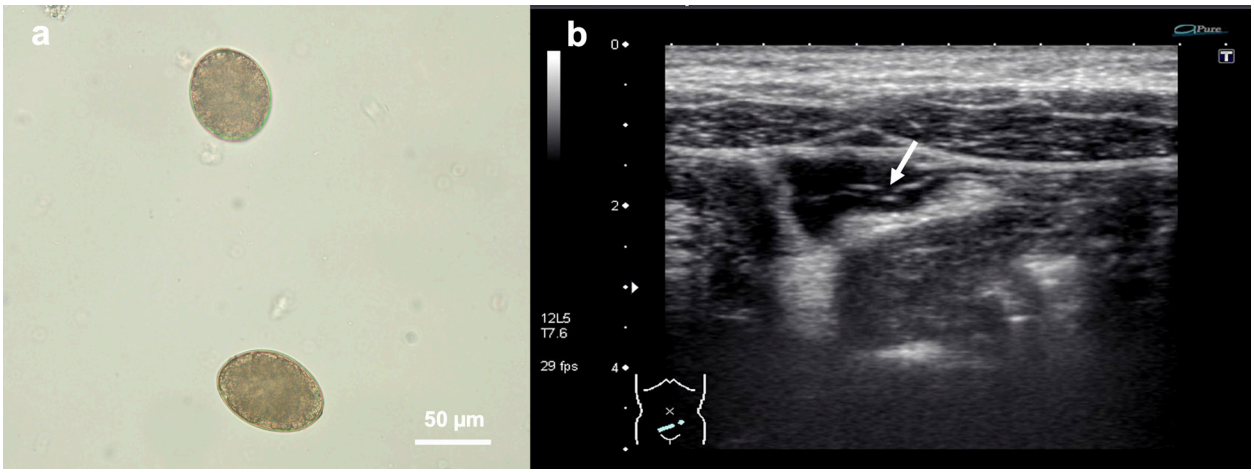
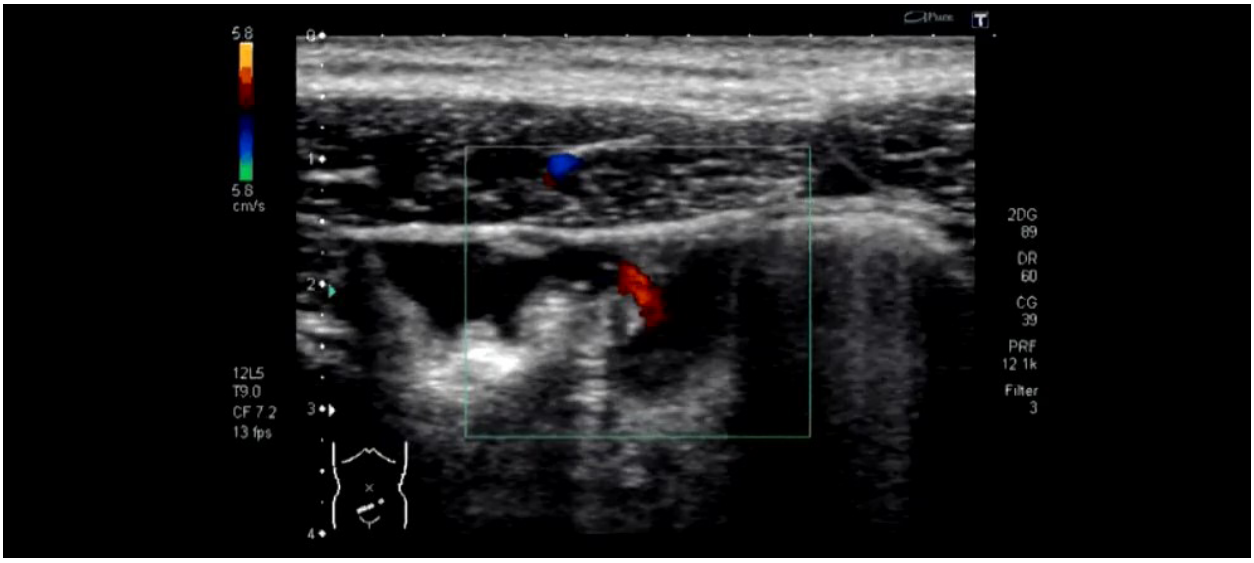


Figure 5. **The broad fish tapeworm *Dibothriocephalus latus* (Linnaeus, 1758) (Cestoda: Diphyllobothriidea)**. (**a**) Eggs of *D. latus*. (**b**) A screenshot of an abdominal ultrasound scan of a patient with *Diphyllobothrium nihonkaiense*, showing the tapeworm as a hyperechoic ribbon-like structure (arrow). (Photo credit: (**a**) Prof. Lin Ai, Chinese Center for Disease Control and Prevention; (**b**) Dr. Hiroki Kitamoto, Kyoto University, Japan)



Extracted still from Video 1: Abdominal ultrasonographic examination of a patient with diphyllobothriasis. A video reveals the tapeworm as a hyperechoic ribbon-like agile structure in the intestinal lumen. (Video credit: Dr. Hiroki Kitamoto, Kyoto University, Japan)

to the intestinal mucosa not only in the terminal ileum [68] and the ileocecal valve [69], but also in the jejunum [70]. This makes it difficult to determine whether further anthelmintic treatment is necessary to eliminate the remaining part of the tapeworm that contains the scolex. Capsule endoscopy, which can detect the proglottids and scolex of the tapeworm in the jejunum, can be used to determine whether additional treatment is needed [70]. Following capsule endoscopy, the patient may need to undergo additional anthelminthic treatment to completely cure diphyllobothriasis and discharge the full tapeworm including scolex.

Hematological and serological analysis can also be performed. Eosinophilia was recorded in a 75-year-old male patient at elevated levels of 2232/µL, compared with a normal range of 100–300/µL [69]. It should be noted that almost any parasitic infection with helminths will lead to the development of eosinophilia, to a greater or lesser extent depending on the parasite and the patient's immune system, and therefore the detection of eosinophilia alone is not a diagnostic parameter. The patient's prior medical history and favored foods (consumption of local or imported raw fish, particularly raw trout and salmon) can provide useful information [69]. Any molecular

diagnostics, such as PCR, although facilitates tapeworm identification, are used mainly for research purposes.

Treatment

Praziquantel, a pyrazinoisoquinoline derivative, is recommended for treating diphyllobothriid tapeworm infections regardless of the species involved. A single oral dose of 15-25 mg/kg^{-1} is usually effective as fecal examination at 2 months post-treatment often shows no evidence of a recurrent infection [6,60]. However, a second dose is required in some patients if the tapeworm is not fully expelled in feces, or in cases of recurrence of diarrhea and re-appearance of eggs on fecal examination. Other drugs such as atabrine, bithionol, and niclosamide have been also used to treat patients with *D. latus* infection [71].

The tapeworm can be manually removed via endoscopy by using a basket retrieval device to grasp and pull the worm out of the bowl through the anus gently to avoid tearing the strobila; however, this does not often succeed in removing the entire tapeworm. An injection of the GI contrast medium diatrizoate (gastrografin) into the third part of the duodenum or jejunum using a duodenal tube, upper GI fiberscope or endoscopy, facilitates the removal of the tapeworm [68,72]. As gastrografin passes rapidly through the small intestine the tapeworm is translocated from the jejunum to the colon by intestinal peristalsis. The tapeworm discharge can be monitored radiologically and may be completed within a short time (~35 minutes) [68]. Using gastrografin can help to remove the tapeworm with an intact scolex which is important in avoiding the recurrence of infection. In a previous study, gastrografin was injected directly into the tapeworm by using an antegrade double-balloon enteroscope. The discharge of the worm into the transverse colon was monitored by fluoroscopic imaging and fecal expulsion of the whole tapeworm (ie, with scolex) occurred after 12 hours [69].

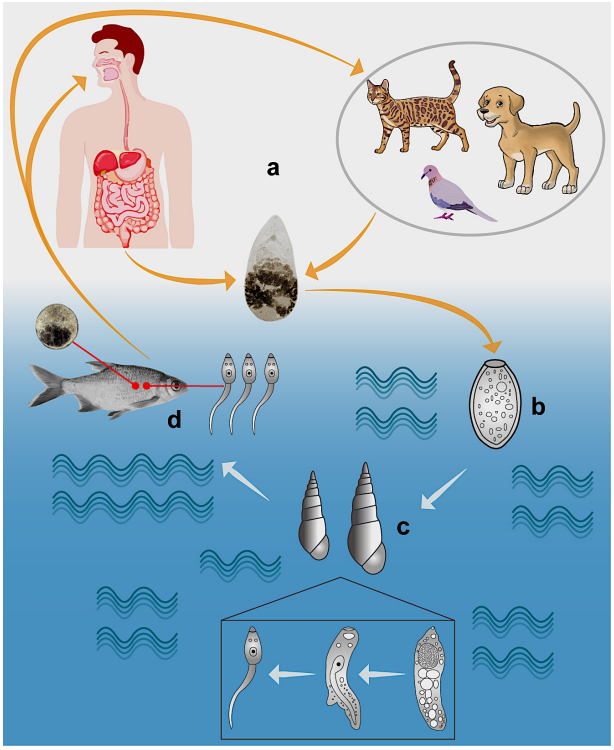


Figure 6. Life cycle of *Metagonimus yokogawai*. (**a**) Adult *M. yokogawai* flukes inhabit the small intestine of humans and release eggs (**b**) that pass in the feces. First larval stage known as miracidia hatch from eggs and infect a molluscan freshwater snail (**c**). The miracidia develop to other developmental forms, ending up with the formation of cercariae, which are expelled from the snails into the water to infect the sweetfish (*Plecoglossus altivelis*), the dace (*Tribolodon* spp.) and the perch fish (*Lateolabrax japonicus*), where they encyst as metacercariae in the fish muscles (**d**). The encysted metacercariae in raw, undercooked, salted or pickled fish must be ingested by fish-eating birds and mammals including humans for the life cycle to be completed. Adapted from the Centers for Disease Control and Prevention DPDx website (https://www.cdc.gov/dpdx/metagonimiasis/index.html)

METAGONIMIASIS

Several zoonotic trematodes can be transmitted through consumption of fish [73]. For example, intestinal infection by *Metagonimus* flukes (Trematoda: Heterophyidae) results in a disease called "metagonimiasis" which is associated with GI manifestations [5]. Five *Metagonimus* species including *M. yokogawai, M. katsuradai*, *M. takahashii*, *M. miyatai*, and *M. minutes* have been reported in humans [74], however, *M. yokogawai* is the most pathogenic species.

Biology and Epidemiology

M. yokogawai has an indirect life cycle as shown in Figure 6. Humans acquire *M. yokogawai* infection by eating infected raw freshwater fish. As with other fish-borne parasitic zoonoses, the risk of infection correlates with the dietary habits of people [5]. Most cases of *M. yokogawai* infections have been reported in East Asia and in the Asian regions of Russia [75,76-78], with prevalence rates particularly high in Japan, Korea, and Taiwan [76,79].

Clinical Symptoms

Adult *M. yokogawai* flukes parasitize the small intestine and cause enteritis. Often, infections are asymptomatic; however, some patients may develop symptomatic illness, which is dependent on the infection dose and immune status of the individual. Clinical features most often associated with *M. yokogawai* infection include

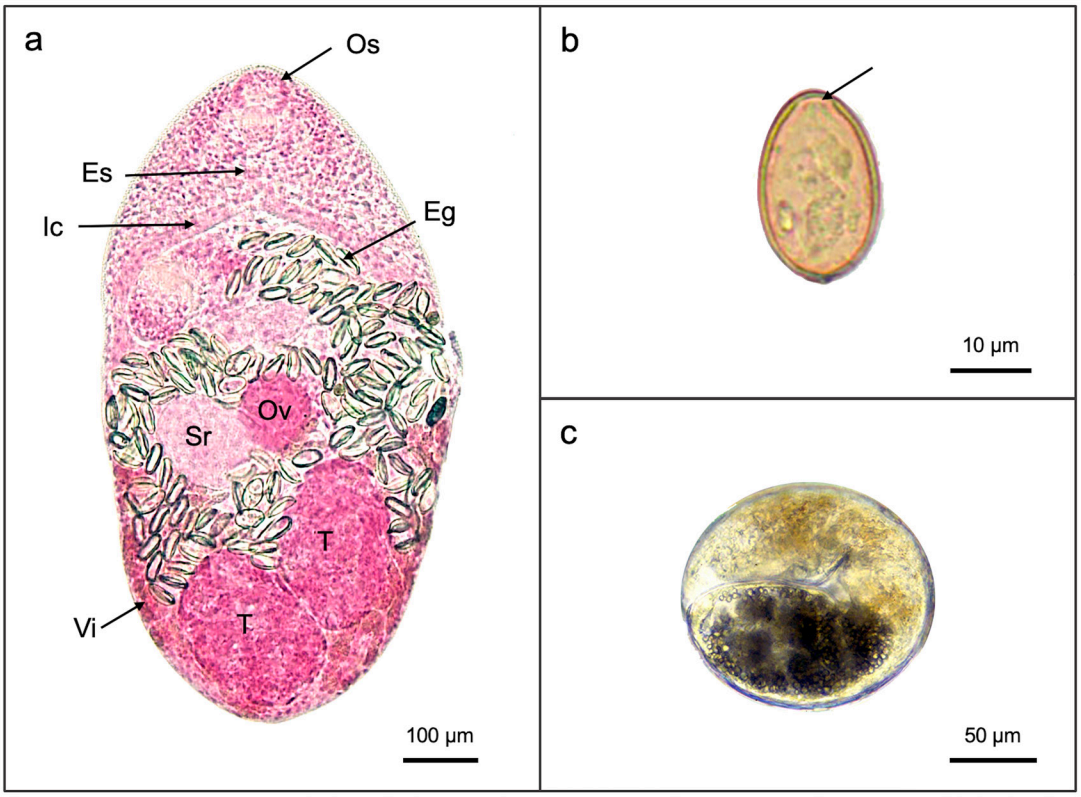


Figure 7. ***Metagonimus yokogawai***. (**a**) Adult fluke. Os, oral sucker; Eg, egg; Es, esophagus; Ic, intestinal caeca; Ov, ovary; Sr, seminal receptable; T, testis; Vi, vitelline follicles. (**b**) Egg of *M. yokogawai* is operculated (arrow), thick-shelled, yellow brown in color and embryonated (contain larva). (**c**) Encysted metacercaria of *M. yokogawai* separated from the muscle of sweetfish (*Plecoglossus altivelis*). (Photo credit: Prof. Jong-Yil Chai, Seoul National University College of Medicine, Korea)

abdominal pain, discomfort, intermittent diarrhea, easy fatigability, weakness, weight loss, and anorexia. Due to the non-specific nature of these symptoms, *M. yokogawai* infection may be overlooked by patients and physicians. *M. yokogawai* infection was associated with brain hemorrhage and diabetes mellitus in one patient [1].

Careful attention should be paid to *M. yokogawai* infection because the small-sized eggs of these intestinal flukes can, in rare cases, gain access to circulation and carried by the blood stream to various extra-intestinal tissues, causing serious complications such as emboli and granulomatous reactions [80,81]. As with other heterophyid infections, *M. yokogawai* is particularly serious especially among immunosuppressed patients, who are at increased risk of invasive erratic infection and can develop severe symptoms when *M. yokogawai* flukes infect unusual sites such as myocardium, brain, and spinal cord.

Diagnosis

Laboratory diagnosis of *M. yokogawai* infection involves the recovery and identification of adult flukes and eggs in the feces. *Metagonimus* spp. are minute (1.5 x 0.5 mm) and have a laterally deviated (sub-medial located) ventral sucker and two testes near the posterior end (Figure 7a). A number of fecal examination methods, including fecal smears and concentration techniques can be performed. Eggs of *M. yokogawai* have dark yellow or brown color, elliptical shape, smooth shell surface, and less prominent operculum (Figure 7b). The specific diagnosis of human *M. yokogawai* infection is problematic because *M. yokogawai* eggs resemble those of other intestinal heterophyid flukes and opisthorchiid liver flukes, which causes hepatobiliary manifestations [79]. The molecular technique PCR has been used to detect heterophyid infections in human feces and to differentiate *M. yokogawai* infection from other heterophyid, *Clonorchis sinensis* or *Haplorchis taichui* infections [82]. Detection of infection in the fish host involves microscopic examination of the fish muscle for encysted metacercariae cysts, which are spherical, or slightly elliptical, and 0.14–0.16 mm in diameter (Figure 7c).

Treatment

The treatment of metagonimiasis includes 20 mg

kg^{-1} of praziquantel as a single oral dose or up to 3 days in heavy infections [83].

PREVENTION AND CONTROL OF FISH-BORNE PARASITIC INFECTIONS

Breaking the parasite's transmission cycle. Certain measures can be employed to disrupt the life cycle of the parasites and prevent infection. For example, the detection and treatment of infected people and establishing infrastructures for the elimination of excreta in homes and population centers are essential for breaking the life cycle of the intestinal flukes and the tapeworms via preventing their first stage larvae from reaching their intermediate hosts. Although it seems unfeasible to control parasite infection in fish, breeding fish in closed farms with minimal or no contact with other hosts involved in the parasite's life cycle can minimize the possibility for transmission of the parasite's developmental stages between fish and these hosts, and thus interrupt the parasite's life cycle.

Increasing consumers' awareness. Fish-borne parasites are transmitted to humans via ingestion of infected raw or undercooked fish, or fish delicacies that have not been sufficiently processed, such as sushi and sashimi. This may result in an increase in the exposure of consumers to fish-borne parasites as fish eaten raw may harbor infective/viable parasite stages [84]. Therefore, a simple but very effective preventive measure to control these parasites is to discourage the people from eating raw fish or fish that have not been properly cooked or frozen [16,39,57]. People should also practice careful food handling, preparation and cooking procedures. Deep-freezing and adequately cooking fish can efficiently inactivate the infective stage of most of the parasites. In all member States of the European Union, regulations dictate that fish or fishery products intended for raw consumption must be frozen at a temperature < −20°C for a minimum of 24 hours; however individual consumers do not always strictly follow these instructions [12].

Inactivation of the infective stages. Fish evisceration soon after capture and thorough cooking of fish are among the most effective measures to reduce the risk of acquiring the infective anisakid larvae [85]. *Anisakis* larvae are deactivated by heating to 60°C for a minimum of one minute [86,87] or freezing at −35°C for ≥15 hours, or at −20°C for a minimum of 24 hours [88-90]. Microwave heating kills *Anisakis* larvae in Arrowtooth flounders [91,92]. However, in domestic microwave ovens (2450 MHz, 700 W) heat may not reach all parts of the fish body, allowing some larvae to remain viable [92]. High-pressure, nonthermal, processing (300 MPa for 5 min) may render *Anisakis* larvae in Mackerel filet nonviable [93]. The minimal effective dose of gamma radiation is >0.1–0.5 kGy for fish parasites, apart from *Anisakis* which has a high radio resistance (10 kGy) [94]. For *D. latus*, freezing at −18°C for ≥24 hours, or cooking at 55°C for ≥5 minutes kills the larvae [95].

For the intestinal flukes, such as *Heterophyes*, temperatures as high as 100°C for >15 minutes are required to inactivate metacercariae in fish [96].

CONCLUSION AND OUTLOOK

Gastrointestinal manifestations associated with fish-borne parasitoses are generally non-specific and may overlap with other infections. Therefore, diagnosis should involve a history of eating raw or lightly cooked fish, physical examination, standardized parasitological examination, and blood testing. Some imaging modalities can improve the diagnosis and management of these diseases, for example endoscopy for gastric anisakiasis and colonoscopy, with gastrografin and antegrade double-balloon enteroscopy, for intestinal diphyllobothriasis. Treatment involves the use of anthelmintic agents, mostly praziquantel to kill *Dibothriocephalus* and *Metagonimus*, and physical extraction of *Anisakis* larvae. Purgatives (eg, saline or magnesium salt) can be administered after anthelmintic treatment to provoke the expulsion of the dead worms with feces.

The worldwide increased prevalence of fish-borne parasitic infections can be attributed to the improved awareness and knowledge of health care workers together with the development of new and better diagnostic techniques that have reduced the underdiagnosis of these infections, bringing to light many more cases that were not diagnosed previously. Also, there is increasing trends of raw fish consumption, which makes controlling fish-borne parasites at the consumer level difficult to achieve. The implementation of more personal and regulatory actions, together with food-safety measures, including inspection of imported and local fish, and fish products will help to support control efforts. Although the detection of these parasites in fish is desirable, it is difficult to implement because of the large volume of fish marketed worldwide. Some techniques have been developed to facilitate the detection of fish-borne parasites, including anisakid L3s, and opisthorchiid and heterophyid metacercariae [97,98], however, their implementation would involve an additional cost that is currently unaffordable for the fishing industry. Given these challenges, a key priority for public health authorities should focus on educating local communities and food handlers on the potential health risk associated with raw fish consumption. People with certain underlying medical illnesses and immunosuppressed individuals are more likely to develop severe illness and should be particularly careful. It is absolutely essential to advise people to modify their habits of eating raw or lightly-cooked fish. Optimal infection control

measures may vary between countries due to differences in the available resources, population size, cultural diversity, health literacy, and political factors.

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