Short Report: Cryptic and Asymptomatic Opisthorchis felineus Infections

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Abstract. We describe the diagnostic difficulties experienced during an opisthorchiasis outbreak. Of 31 infected individuals, 61.3% were asymptomatic, and in the 12 symptomatic individuals, the duration of non-pathognomonic symptoms was shorter than 4 weeks. Serology by enzyme-linked immunosorbent assay and polymerase chain reaction fecal analysis were shown to be the most sensitive diagnostic tools.

Opisthorchiasis and clonorchiasis are zoonotic infections caused by liver flukes of the genera Opisthorchis and Clonorchis reported in Asia and Europe, where they affect an estimated 10 million people.1 However, most of the cases are from Asia, where the etiological agents are O. viverrini and C. sinensis. In Europe, the etiological agent is O. felineus, and cases of human infection have been documented in Byelorussia, Germany, Greece, Italy, Lithuania, Poland, Romania, Russia, Spain, and the Ukraine.2–5 Humans acquire the infection by consuming raw freshwater fish of the family Cyprinidae, which harbor the larval stage, metacercariae, in their muscles.6 Because the clinical picture of infection depends on the number of fluke worms, persons who ingest a low number of parasites may have only mild or asymptomatic infection,6,7 which could result in delayed or missed diagnosis. In fact, fluke parasites cannot multiply in the human body, and therefore, high fluke infection intensities can only be reached by repeated exposure to raw fish consumption. The importance of a timely diagnosis is in the fact that untreated infection can result in severe complications. In fact, in Asia, opisthorchiasis and clonorchiasis caused by a great number of O. viverrini and C. sinensis worms have been shown to cause cholangiocarcinoma, which can develop even 20 years after infection.1 In Italy, opisthorchiasis in humans was first reported in 2003, and since that time, a number of outbreaks have occurred.5,8 In the present study, we describe an outbreak that occurred in 2009 to illustrate how diagnosis can be delayed when a high proportion of individuals have non-specific symptoms or no symptoms at all.

A case of opisthorchiasis was defined as the presence of opisthorchiidae eggs in feces and/or anti-Opisthorchis immunglobulin G (IgG) in serum of persons who had consumed raw freshwater fish. Eggs were searched for in fecal samples after formol-ether concentration using a light microscope (150–400× magnification). The fecal sediment was preserved in absolute ethyl alcohol for the molecular diagnosis and identification of the parasite. Parasite DNA was amplified by polymerase chain reaction (PCR) performed according to a published protocol.9 The 250-bp amplicon was sequenced and compared with the internal transcribed spacer 2 (ITS2) sequences of O. felineus, O. viverrini, and C. sinensis present in the GenBank database. Serology was performed with enzyme-linked immunosorbent assay (ELISA) in accordance with a standard protocol using excretory/secretory (E/S) antigens from adult worms of O. felineus maintained in vitro.

On March 15, 2010, a 46-year-old woman who organized gastronomic events presented with eosinophilia (4,200 cells/µL) to the Infectious Disease Unit of Belcolle Hospital, Viterbo (Latium Region, central Italy). She had suffered fever, headache, muscle and abdomen pain, asthenia, and general malaise between late December and January. In early January, liver ultrasonography had shown gallstones, and laboratory tests revealed marked leukocytosis (26,700 cells/µL) with eosinophilia (18,600 cells/µL). At the time, she was treated with mebendazole (200 mg daily for 3 days), but there was no reduction in the eosinophilia. A surgeon had suggested that the eosinophilia was related to gallstones; however, because a hematologist had suggested that it was possibly of infectious origin, the woman presented to the Belcolle Hospital. A fecal sample tested positive for Opisthorchis sp. eggs, and a serum sample tested positive for anti-Opisthorchis IgG (optical density [OD] = 1.720; cutoff = 0.280). The woman reported that she had consumed raw fillet of tench (Tinca tinca) during a gastronomic event in the town of Bomarzo (Viterbo province, Latium region) on December 8, 2009. She also reported that her colleague had become ill; in late December of 2009, the colleague, according to medical records from another healthcare facility, had leukocytosis (24,000 cells/µL) and eosinophilia (10,100 cells/µL) and had been diagnosed with an allergy of unknown etiology.

Because the gastronomic event was by invitation only, it was possible to trace all 44 participants (April to May of 2010, 4–5 months after the event). Twelve participants had had symptoms of unknown etiology in late December of 2009 and early January of 2010 (Table 1). Of the 44 serum samples taken, 31 samples (23 males and 8 females; average age = 44.9 years, range = 27–69 years) tested positive for anti-Opisthorchis IgG (average OD = 1.465, range = 0.545–2.238) for an attack rate of 70.4%. Of the 36 fecal samples taken, 28 samples tested positive by either microscopic analysis (N = 19) or PCR (N = 9). Three persons with stools that were negative for Opisthorchis sp. by both microscopic and PCR analyses showed OD values of 1.259, 1.579, and 2.063 by ELISA and 3.500, 1.600 and 4.200 eosinophils/µL, respectively. At diagnosis (4–5 months post-infection), eosinophilia, elevated transaminase levels, and elevated γ-glutamyl transpeptidase (GGT) levels were detected in 25.8%, 13%, and 37.9% of the infected persons, respectively (Table 1). Twenty-eight individuals were treated immediately with albendazole (10 mg/kg daily in two doses for 7 days); the remaining three individuals were treated with praziquantel.
Although the OD values (average GGT values 69, 83, and 84 U/L) were still present in another was present only in the index patient, and higher-than-normal values had decreased to 1.246 and 1.059, respectively, but no eggs were detected in the feces. By contrast, eggs were still present in the fecal sample of the index patient, who had failed.

The epidemiological investigation revealed that the organizers of the gastronomic event had used a catering company that had prepared marinated fillets of tench purchased at the fish market of Lake Bolsena (Latium region, central Italy). The PCR amplicons obtained from the fecal samples were sequenced and identified as belonging to O. felineus and bile duct cancer has never been shown in humans, in the area of Tyumen oblast (Russia), the highest incidence of cholangiocarcinoma in humans was found in the same area as the highest incidence of O. felineus infection. Moreover, in experimentally infected hamsters, O. felineus is more pathogenic than O. viverrini.

Furthermore, in mild infections, the clinical pattern is cryptic, and laboratory parameters such as eosinophilia, GGT, and transaminases can return to normal or close to normal even when adult worms are still alive in the bile duct and shedding eggs. In fact, in the outbreak described herein, 21 people with a normal eosinophil count, 16 people with normal GGT values, and 25 people with normal transaminase values shed O. felineus eggs 4–5 months post-infection. The only laboratory feature that allows a correct diagnosis to be made is the detection of anti-Opisthorchis IgG in the serum, which is detectable for a long period of time and decreases very slowly only after successful treatment.

The microscope detection of Opisthorchidae eggs in feces, bile, or duodenal fluid, which is non-invasive and inexpensive, is considered the gold standard for diagnosis in laboratories that cannot use an in-house serology or molecular analysis; however, the level of experience of the individual performing the analysis plays a key role in the diagnosis. In fact, fecal samples from the 12 symptomatic people were considered to be negative for helminthic eggs in private laboratories.

This outbreak has taught us several lessons. (1) The consumption (even single consumption) of raw fish dishes can lead to trematodiases in Europe and the Western world. (2) Symptoms disappear quickly. (3) Laboratory measurements rapidly return to normal values. (4) Opisthorchiasis does not show pathognomonic signs or symptoms in mild infections. (5) In the absence of successful treatment, infected individuals can recover from clinical disease but still harbor adult worms.
and possibly develop complications. (6) Repeated stool examination is required in individuals with low infection intensities to detect eggs. (7) In non-endemic regions, physicians can have serious problems in performing differential diagnoses. (8) At least in the European market, there is a lack of diagnostic kits for detecting anti-Opisthorchis IgG in serum and Opisthorchis DNA in stools. (9) Proper freezing of fish intended to be consumed raw is not always used to protect the consumers.

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