Short Report: Cryptic and Asymptomatic *Opisthorchis felinus* Infections

Orlando Armignacco, Fabrizio Ferri, Maria Angeles Gomez-Morales, Luciano Caterini, and Edoardo Pozio*

Unità Operativa Complessa Malattie Infettive, Belcolle Hospital, Viterbo, Italy; Department of Infectious,
Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy

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. felinus*, 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 opisthorchidae eggs in feces and/or anti-*Opisthorchis* immunoglobulin 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. felinus*, *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. felinus* maintained *in vitro*.

On March 15, 2010, a 46-year-old woman who organized gastronomic events presented with eosinophilia (4,200 cells/ μ L) 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/ μ L) with eosinophilia (18,600 cells/ μ L). 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/ μ L) and eosinophilia (10,100 cells/ μ L) 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/ μ L, 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

*Address correspondence to Edoardo Pozio, Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, viale Regina Elena 299, Rome 00161, Italy. E-mail: edoardo.pozio@iss.it

TABLE 1
Clinical signs and symptoms and laboratory features of the 31 people who acquired opisthorchiasis

Reported signs and symptoms* (1 month post-infection)	Number of patients (%)
Fever	12† (38.7)
Pain in the muscles	10 (32.5)
Abdominal pain	5 (16.1)
Asthenia and general malaise	12‡ (38.7)
Back pain with headache	4 (12.9)
Laboratory features of former 12 symptomatic people 4–5 months post-infection	
Eosinophilia (> 500/μL)	2 (930/μL, 4,200/μL)
Transaminases (> 41 U/L)	4 (range = 46–80 U/L)
GGT (> 55 U/L)	5 (range = 65–303 U/L)
Laboratory features of all 31 infected people 4–5 months post-infection	
Eosinophilia (> 500/μL)	8 (25.8; average = 1,792/μL; range = 670–4,200/μL)
Transaminases (> 41 U/L)	4 (12.9; range = 46–80 U/L)
GGT (> 55 U/L)	11 (35.4; average = 129; range 65–303 U/L)
Anti- <i>Opisthorchis</i> IgG	31 (100)
<i>O. felineus</i> eggs and/or DNA in feces	28 (90.3)

*Of the 31 people who acquired opisthorchiasis.

†Three patients had fevers up to 39°C.

‡The duration of asthenia was up to 4 weeks in three patients.

(25 mg/kg three times [TID] for 1 day), which is the drug of choice but not commercially available in Italy.

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*.

Between 3 and 8 months after treatment, 21 (67.7%) diagnosed individuals returned for follow-up. Eosinophilia (640/μL) was present only in the index patient, and higher-than-normal GGT values (69, 83, and 84 U/L) were still present in another three persons. All 21 individuals had positive serology, although the OD values (average = 0.819, range = 0.244–2.047) were lower than those values detected at diagnosis; 7 months later (10–15 months after treatment), two of the individuals who still had high OD values (2.047 and 1.492) were treated again with praziquantel. Two years after the infection, the OD 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 an OD value of 0.691 by ELISA, suggesting that treatment had failed.

The experience of the individuals who acquired opisthorchiasis at the gastronomic event and had definitive diagnosis made only 5 months after infection revealed that physicians are unfamiliar with this disease and do not even consider it when attempting to perform a diagnosis. The diagnosis was complicated by the simultaneous occurrence of the peak of influenza in the winter of 2009 and 2010.

In the six outbreaks that have occurred in Italy since 2003, the number of asymptomatic persons ranged from 17.8–100% (61.3% in the outbreak described herein) (Pozio E, unpublished data).^{5,8,10} The high percentage of asymptomatic persons increases the risk of underdiagnosis, resulting in chronic infections, which could cause histopathological changes ranging from inflammation to precancerous lesions (cholangiofibrosis), which was observed in hamsters.¹¹ *O. viverrini* and *C. sinensis* are classified as group 1 carcinogens by the International Agency for Research on Cancer.¹ However, it should be

stressed that morbidity and severe complications (e.g., the development of cholangiocarcinoma) are a consequence of longstanding infections with high worm loads. Single exposures and the resulting worm infection intensity bear a much lower risk of severe morbidity and/or severe complications. Although a direct relationship between opisthorchiasis caused by *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.¹² Moreover, in experimentally infected hamsters, *O. felineus* is more pathogenic than *O. viverrini*.¹¹

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 trematodiasis 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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Authors' addresses: Orlando Armignacco, Fabrizio Ferri, and Luciano Caterini, Unità Operativa Complessa Malattie Infettive, Belcolle Hospital, Viterbo, Italy, E-mails: armignacco@asl.vt.it, faberferri@gmail.com, and lucianocaterini@libero.it. Maria Angeles Gomez-Morales and Edoardo Pozio, Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy, E-mails: mariaangeles.gomezmorales@iss.it and edoardo.pozio@iss.it.

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