

# A large outbreak of *Opisthorchis felineus* in Italy suggests that opisthorchiasis develops as a febrile eosinophilic syndrome with cholestasis rather than a hepatitis-like syndrome

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**Abstract** We describe the greatest Italian human acute opisthorchiasis outbreak acquired from eating raw tenches. Out of 52 people with suspected opisthorchiasis, 45 resulted in being infected. The most frequent symptoms and laboratory findings were fever, abdominal pain and eosinophilia. Seven tri-phasic computed tomography (CT) scans were done, showing multiple hypodense nodules with hyper-enhancement in the arterial phase. All patients took one day of praziquantel 25 mg/kg TID without failures. Reported symptoms suggested a febrile eosinophilic syndrome with cholestasis rather than a hepatitis-like

syndrome. It seems common to find hepatic imaging alterations during acute opisthorchiasis: CT scan could be the most suitable imaging examination. Even if stool test remains the diagnostic gold standard, we found earlier positivity with the serum antibody test. Without previous freezing, the consumption of raw freshwater fish should be avoided.

## Introduction

*Opisthorchis felineus* is a trematode transmitted to human beings through the ingestion of contaminated raw freshwater fish of the family Cyprinidae. In Europe, a high prevalence of human infection has been reported in Ukraine, Russia and Byelorussia [1]. In Italy, in the last eight years, two large and two small outbreaks of acute human infections were reported in persons who had eaten raw freshwater fish from Central Italy [2, 3]. The aim of this report is to describe the largest human outbreak of opisthorchiasis which occurred in a non-endemic region of Northern Italy after the consumption of tenches fished in a Central Italy lake.

## Materials and methods

This was an observational study on people living in the Valle d'Aosta region (North-Western Italy) who ate tench tartare (*Tinca tinca*) infected with *O. felineus* metacercariae during a public dinner held in Challand-Saint-Anselme on 24th July 2010. The first patient was hospitalised on 15th August and ten additional people were observed from 22nd to 26th August. After the identification of the index patients

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a case of opisthorchiasis in both symptomatic and asymptomatic persons who had attended the dinner (exposed people) was defined as: (1) the presence of increased values of liver enzymes (alkaline phosphatase, ALP; gamma-glutamyl-transpeptidase, GGT) and eosinophilia ( $>500$  cells/mm<sup>3</sup>) in the absence of other pathologies; and/or (2) the detection of Opisthorchidae eggs or their DNA in a stool sample by microscope or polymerase chain reaction (PCR), respectively; and/or (3) the detection of anti-Opisthorchidae IgG in the serum.

In addition to the first 11 patients (index patients), after 26th August, we observed as outpatients another 41 individuals who had attended the dinner.

Follow-up visits were scheduled at 15, 30, 60 and 90 days after treatment. The microbiological diagnosis was based on direct stool examination after formol-ether concentration and serology. The molecular identification of parasites in the faecal samples was carried out by PCR from concentrated stool samples [3]. Two serological tests were used: a commercial kit to detect anti-digenea trematode antibodies based on the haemoagglutination technique (HA, Distomatose Fumouze®, Fumouze Diagnostics, Levallois Perret, France; specificity 96.6%; sensitivity 96.1%; cut-off  $\geq 1/160$ ); and a home-made enzyme-linked immunosorbent assay (ELISA) using excretory/secretory antigens of *O. felineus* adult worms to detect anti-Opisthorchidae IgG (specificity 96.3%; sensitivity 98.5%; serum dilution 1/100) [3].

## Results

Out of 93 people who had attended the dinner, 52 inhabitants of the Valle d'Aosta region were followed as outpatients or inpatients between 15th August 2010 and 31st December 2010 (29 females and 23 males, median age 51.5 years, range 9–84 years) (Table 1). The other 41 individuals who attended the dinner but who were resident in other Italian regions (40) or outside Italy (1) were traced and referred to local hospitals. We were informed that 15 of them (14 in Italy and one in the Netherlands) turned out to be infected by *O. felineus*. Forty-five patients of the Valle d'Aosta region met the definition of opisthorchiasis (attack rate 86.5%) and eight of them (17.7%) were hospitalised due to high fever for an average of 6.6 days (range 3–12 days). Thirty-seven patients (82%) were symptomatic with eosinophilia and increased liver enzymes (Table 1). The most common signs and symptoms are shown in Table 2. Less frequent signs and symptoms were: dry cough, skin rash, lack of appetite, lipothymia and constipation. No case of jaundice was observed. The specific clinical signs and symptoms of five patients with eggs in stool and a positive serology are shown in Table 3. The median onset of signs and symptoms was 20 days after infection (range 7–37 days). Most of the infected people (81%) were symptomatic from the third and fourth week after infection, but four people (11%) developed symptoms as early as after 7 days and other three people (8%) at the

**Table 1** Clinical and laboratory findings of the 52 people of the Valle d'Aosta region who were followed as outpatients (44) or inpatients (8) at the Infectious Diseases Unit, "U. Parini" Hospital, Aosta, Italy, between 15th August and 16th September 2010

No. of people	Clinical signs and symptoms	Eosinophilia $>500/\text{mm}^3$	Altered liver enzymes <sup>a</sup>	Eggs in stool <sup>b</sup>	ELISA	HA	Diagnosis of opisthorchiasis
2 <sup>e</sup>	yes	yes	yes	yes	nd <sup>d</sup>	pos	yes
2 <sup>e</sup>	yes	yes	yes	no	pos	pos	yes
4 <sup>f</sup>	yes	yes	yes	no	pos	nd	yes
3	yes	yes	yes	yes	pos	nd	yes
5	yes	yes	yes	yes	nd	nd	yes
2	no	yes	yes	yes <sup>c</sup>	nd	nd	yes
3	no	no	no	no	nd	pos	yes
21 <sup>f</sup>	yes	yes	yes	no	nd	nd	yes
3	no	yes	yes	no	nd	nd	yes
7	no	no	no	no	nd	neg	no

<sup>a</sup> Gamma-glutamyl-transpeptidase  $>40$  U/L; alkaline phosphatase  $>40$  U/L

<sup>b</sup> Detected by microscopy

<sup>c</sup> One was microscopically negative but polymerase chain reaction (PCR)-positive

<sup>d</sup> nd = not done

<sup>e</sup> Hospitalised patients

<sup>f</sup> Two were hospitalised

**Table 2** Main signs and symptoms observed in infected patients ( $n = 45$ )

Symptoms	No. of patients (%)
Fever	29 (64)
Abdominal pain	22 (49)
Headache	16 (36)
Asthaenia	14 (31)
Arthralgia	12 (27)
Diarrhoea	10 (23)
Nausea	8 (18)

end of the fifth week. Eight patients were asymptomatic and, among them, five had eosinophilia ( $>1,000/\text{mm}^3$ ) and increased liver enzymes and three with no eosinophilia or altered liver enzymes, showed a positive serology. The baseline laboratory features of infected people are shown in Table 4. The median onset of laboratory abnormalities was 31 days (range 20–50 days). A correlation between the severity of symptoms and the degree of eosinophilia and cholestasis was observed in 50% of infected people.

By an abdominal ultrasonography (US) scan, all eight inpatients displayed unspecific findings, whereas by an abdominal tri-phasic computed tomography (CT) scan, which was used for seven of the patients, multiple hypodense nodules (diameter 2.5–5.0 cm) with hyper-enhancement in the arterial phase were observed (Fig. 1). One inpatient underwent a liver biopsy showing an acute inflammatory disease with dilatation of portal spaces and granulocyte infiltration due mostly to eosinophils and, to a lesser extent, lymphocytes and monocytes (Fig. 2).

Out of the 52 stool samples tested, ten were positive by microscopy for Opisthorchidae eggs and two were positive by PCR out of the six tested. The first direct stool examination was made three weeks after infection, but the

**Table 4** Laboratory features of infected patients ( $n = 45$ ) before treatment

Laboratory feature	Median (range)	No. of patients (%)
WBC $>10,500/\text{mm}^3$	13,775 (10,706–35,300)	21 (47)
Eosinophils $>500/\text{mm}^3$	2,400 (600–25,063)	42 (93)
CRP $>0.5$ mg/dl	1.85 (0.5–9)	39 (87)
GGT $>40$ U/L	180 (52–556)	35 (78)
ALP $>40$ U/L	940 (778–1,410)	30 (67)
ALT $>37$ U/L	102 (43–148)	29 (64)
AST $>37$ U/L	55 (38–155)	19 (42)

WBC: white blood cells; CRP: C-reactive protein; GGT: gamma-glutamyl-transpeptidase; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase

first positive stool sample was detected only five weeks after infection, in spite of the stool samples being tested every 3–4 days. Four weeks after infection, all of the nine people tested by ELISA resulted positive, whereas only 7 of the 14 people tested by HA resulted positive; the seven people who tested negative by HA did not show any clinical signs or symptoms, no eosinophilia or altered liver enzymes, and their stool samples tested negative. The epidemiological investigation showed that the source of infection was tartare from tenches fished in Lake Bracciano (Central Italy).

All infected patients were treated with praziquantel 75 mg/kg in three doses for one day immediately after the diagnosis. A quick response (resolution of fever) was observed after 24–48 h from the onset of treatment. The drug was well tolerated and a second dose was given to only two patients because of suspected low absorption due to vomiting.

Twenty-eight infected people (62%) attended the first follow-up 15 days after treatment; of them, 11 (39%) people had a normal eosinophil count, 12 (42%) had

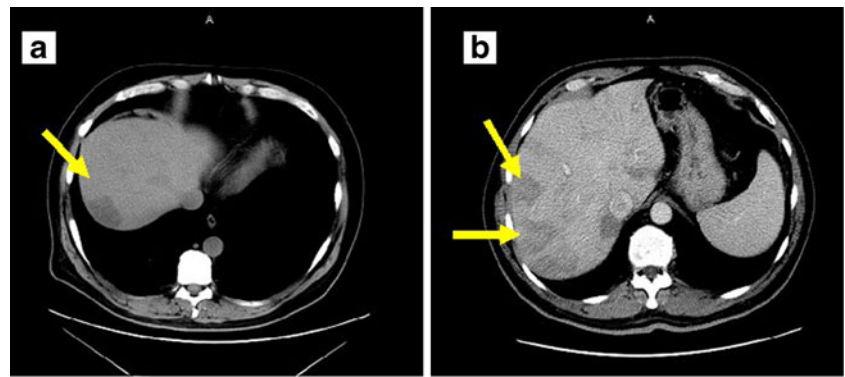
**Table 3** Clinical and laboratory findings of the five patients who were positive either by serology, eggs in stools or polymerase chain reaction (PCR)

Signs and symptoms	Eosinophils up to ( $\text{mm}^3$ )	Liver enzymes <sup>a</sup>				Eggs in stool	ELISA	HA
		ALP	GGT	AST	ALT			
Fever, headache, dry cough, moderate abdominal pain	11,773	873	461	94	121	yes	nd <sup>b</sup>	pos
Fever, headache, dry cough, moderate abdominal pain	25,063	940	330	39	100	yes	nd	pos
Fever, asthaenia	3,113	1,262	349	85	125	yes	pos	nd
Fever, headache, constipation, lipothymia	11,060	981	332	63	135	yes	pos	nd
Fever, headache, diarrhoea	3,500	1,410	362	112	142	yes	pos	nd

<sup>a</sup> ALP: alkaline phosphatase (normal value  $\leq 40$  U/L); GGT: gamma-glutamyl-transpeptidase (normal value  $\leq 40$  U/L); AST: aspartate aminotransferase (normal value  $\leq 37$  U/L); ALT: alanine aminotransferase (normal value  $\leq 37$  U/L)

<sup>b</sup> nd = not done

**Fig. 1** Multiple nodules in the liver by abdominal tri-phasic computed tomography (CT) scan. **a** A 47-year-old man. **b** A 48-year-old man



normal gallbladder enzymes and 6 (21%) had both normal eosinophils and normal gallbladder enzymes. Thirty and 60 days after treatment, 25 (56%) patients attended the second and third follow-up, respectively: 20 (80%) people had normal values for both eosinophils and gallbladder enzymes. At the 90-day follow-up, all of the 22 (49%) patients had normal eosinophil and gallbladder enzyme values. No positive stool was found during the follow-up.

The serological follow-up carried out by HA three months after the praziquantel therapy showed a significant reduction in antibody titres in two patients, a complete negativity in another two, while three patients were lost at the follow-up.

## Discussion

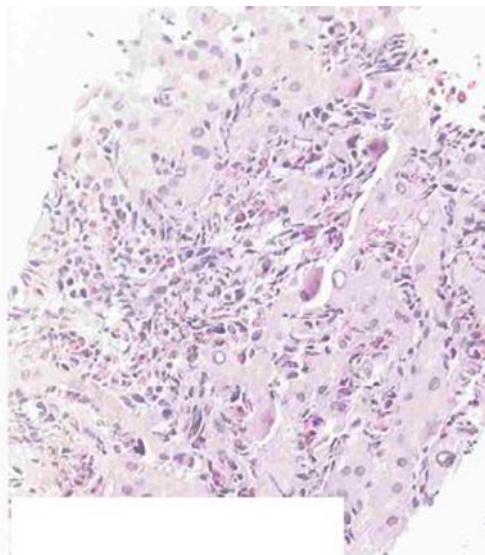
In Italy, two large and two small outbreaks of *O. felineus* infections have been described in the last eight years [3]. In

the European Union, sporadic infections have been reported in Greece and in Germany [4, 5]. The acute infection is described as an association of fever, cutaneous rash, abdominal pain, diarrhoea, nausea and vomiting, with or without jaundice. These signs and symptoms, associated to eosinophilia and increased liver enzymes, have defined the acute *O. felineus* infection as an eosinophilic hepatitis-like syndrome. In the present outbreak, the majority of the patients were symptomatic (82%) and the most common symptoms were high and long-lasting fever, headache and unspecific abdominal pain. Moreover, in the course of this outbreak, gallbladder enzymes were significantly high (with normal bilirubin levels), while circulating hepatic necrosis enzymes were only marginally altered. These results are more characteristic of a febrile syndrome with eosinophilia and cholestasis rather than a hepatitis-like syndrome. The attack rate of the infection (86.5%) is similar to that observed in other outbreaks in Italy [3].

In the 11 index patients, diagnosis had been confirmed by the detection of Opisthorchidae eggs in stools and/or by serology, and eosinophilia and cholestasis indexes were always altered. Based on these findings, it was believed that the diagnosis of opisthorchiasis in an exposed person could be based on eosinophilia and altered cholestasis indexes. Consequently, serology was used successively only for the diagnosis of persons without eosinophilia and altered cholestasis indexes.

Three patients developed symptoms as late as after 5 weeks from consumption and, in one case, laboratory abnormalities were observed only after 50 days, suggesting the need for a long observation period before excluding the presence of infection. In fact, in three asymptomatic patients without cholestasis or eosinophilia, the diagnosis was based on serology.

Many reports describe radiological aspects of chronic Opisthorchidae infection, but little is known about acute infection. There is only one case report of multiple hepatic nodules in an acute infection due to *Clonorchis sinensis* in Taiwan, with CT scan findings similar to those of our patients [6]. The authors concluded that these findings were



**Fig. 2** Histology of a hepatic nodule: eosinophil infiltration in the portal area (magnification 200×)

extremely rare during acute opisthorchiasis. To our knowledge, no CT scan imaging data from acute infections caused by *O. felineus* have been published so far. In this outbreak, hepatic imaging alterations do not seem to be rare during acute *O. felineus* infection and CT scan may be considered as the most appropriate radiological technique.

No clinical failure occurred after the praziquantel treatment, it was well tolerated in all but two people and a quick resolution of the fever, other signs and symptoms, cholestasis and hypereosinophilia was observed. In the majority of patients, no abnormal values of laboratory features were observed at the follow-up.

According to the literature, metacercariae in fish may be killed by freezing at  $-10^{\circ}\text{C}$  for 5–7 days or at  $-28^{\circ}\text{C}$  for 24 h, depending on the size of the fish [7]. In 2008, the Italian Ministry of Health officially recommended to control the fish market of the Cyprinidae family by a warning label “to be eaten after cooking or to be frozen at  $-20^{\circ}\text{C}$  for 7 days”. In the present outbreak, the tenches had been served without being previously frozen, because between Lake Bracciano, from where they had been fished, through three markets (Viterbo, Rovigo, Bolzano) before arriving in Valle d’Aosta, the warning label had been lost.

## Conclusions

The acute opisthorchiasis infection is a clinical syndrome characterised by high fever, headache and unspecific abdominal pain associated with leukocytosis, marked eosinophilia and high values of gallbladder enzymes. Exposed but asymptomatic people without eosinophilia or cholestasis and a negative stool test should undergo a prolonged clinical observation (two months) and antibodies should be tested before excluding the infection. Serology is useful for the diagnosis in the early phase of the disease and the antibody titres that decrease after treatment may be a

marker of the clinical recovery. The tri-phasic computed tomography (CT) scan seems to be more sensitive than the ultrasonography (US) scan to detect, in the early phase, the signs of an acute hepatic infection. Praziquantel leads to a very rapid resolution of the clinical signs and symptoms, cholestasis and hypereosinophilia. Thus, the response to treatment may be considered an “ex adjuvantibus criteria” in exposed patients to make the diagnosis.

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