Research Article

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Behaviour and eating habits as determinants for human opisthorchiasis in the Bolsena Lake area, Italy

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Abstract: In Italy, food-borne outbreaks of opisthorchiasis have occurred since 2003, all of them originating from some lakes in Central Italy where the only European liver fluke, *Opisthorchis felinus* (Rivolta, 1884) is endemic. The aim of the present study is to investigate the factors that may facilitate the life cycle of *O. felinus* in the area of Bolsena Lake, the knowledge of the local population about opisthorchiasis, and preventative healthcare measures. We performed a descriptive observational study through the submission of questionnaires to three target populations: fishermen who fish exclusively in Bolsena Lake, restaurateurs whose businesses are close to the lake and general population living in the province of Viterbo. Results showed hazardous behaviours and eating habits in the three target populations, as well as a poor knowledge of opisthorchiasis and preventative healthcare measures.

Keywords: *Opisthorchis felinus*, questionnaire, risk factors

The most common human liver flukes belong to the family Opisthorchiidae. They share similar life cycles with epidemiology defined by the aquatic environment of their intermediate snail and fish hosts. The main causative agents of human infections are *Clonorchis sinensis* (Cobbold, 1875), *Opisthorchis felinus* (Rivolta, 1884) and *Opisthorchis viverrini* (Poirier, 1886) (Jong-Yil et al. 2005). *Opisthorchis felinus* is the only human infecting trematode of the Opisthorchiidae present in Europe. After the passage of immature stages in fresh water snails (first intermediate host), the cercariae of *O. felinus* emerge from the snail and actively seek a fish of the family Cyprinidae, the second intermediate host (Hering-Hagenbec and Schuster 1996). The fish hosts act as a source of infection for the natural definitive hosts, i.e. fish-eating mammals and humans (Dorny et al. 2009). Humans can be infected by eating raw, inadequately heated or minimally processed freshwater fish (e.g. marinated, smoked, pickled or dehydrated fish products) that harbour the metacercariae (Jong-Yil et al. 2005).

In the acute stage of infection, patients can develop hepatitis-like symptoms with fever, pain in the right upper abdominal quadrant, nausea and emesis. Chronic signs usually include inflammation and fibrosis of the upper biliary tract and can lead to biliary obstruction, liver abscesses, pancreatitis, suppurative cholangitis, portal hypertension and cirrhosis (Keiser and Utzinger 2009).

The first human infections by *O. felinus* in Italy were recorded in 2003 when a foodborne outbreak occurred in the Umbria region (Crotti et al. 2004). From 2003 to 2011 eight outbreaks and four sporadic cases were reported, with a total of 211 confirmed infections. All of the infections were due to the consumption of raw tench *Tinca tinca* (Linnaeus) caught in the Bolsena and Bracciano Lakes (Pozio et al. 2013). There was no evidence of further sources of infection.

Bolsena, Bracciano and Vico Lakes are volcanic lakes in the Lazio Region, where *O. felinus* is endemic. Snails of the genus *Bithynia* Leach have been identified as the first intermediate hosts, whereas tench and cats were identified as second intermediate and definitive hosts, respectively (De Liberato et al. 2011). Currently, no other notifications of the presence of *O. felinus* in lakes and rivers from other Italian regions have been reported.

The aim of the present study was to investigate the local determinants that may facilitate and maintain the life cycle of *O. felinus* and increase the exposure of the human population near Bolsena Lake to the parasite.

MATERIALS AND METHODS

Observational descriptive studies with convenience sampling were performed on the following study populations:

(1) Fishermen operating in Bolsena Lake (Primary Production Operators);

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(2) Restaurateurs whose businesses are close to Bolsena Lake (Post Primary Production Operators).

(3) General population living in the province of Viterbo.

We set up three structured questionnaires, defined as Questionnaire A (to population 1), B (to population 2) and C (to population 3), in order to collect qualitative and quantitative data on these populations. All of the interviewed people were of unknown opisthorchiasis status.

The questionnaires were subdivided into two sections, with the first section common for all three populations. The other sections were specific for each target population. The questions in the first section aimed to investigate:

(i) pet management (dogs, cats or other animals) and, specifically, the habits that may favour the maintenance of the *O. felineus* life cycle;

(ii) the level of knowledge among the target population about the parasite's life cycle and related health risks;

(iii) awareness of earlier outbreaks of opisthorchiasis and the subsequent prevention measures adopted.

The question format was both closed-ended and open-ended. A closed-ended question provides a set of responses or options for which a respondent indicates his choice. In order to standardise the results, closed-ended questions were preferred to open-ended ones. However, the multiple choices of some questions also included the option "other" in which the respondent could specify in an open format a response that was not included. To ensure that our questions addressed our needs, we selected the wording appropriate to the understanding of people with the lowest level of education (Caselli and Zerbi 2005). We administered the questionnaire face-to-face. An interviewer presented the items orally and assisted the respondent of each target population in understanding the questions.

Using Questionnaire A, we interviewed members of two local professional cooperatives for the management of hatcheries (Primary Producers), which ensure artificial reproduction of some freshwater species from Bolsena Lake. The interviews were carried out on randomly selected days of the week from November 2009 to February 2011.

An interviewer presented questionnaire B to operators from the restaurateurs (Post Primary Producers) who were randomly selected from the official food Business Operators' database set up in accordance with EU legislation. The interviews were carried out on randomly selected days of the week from April 2009 to February 2011.

Finally, Questionnaire C was submitted to attendees at Viterbo's Public Health Surgery, a health facility where doctors provide general advice on health prevention and perform routine vaccination for the general resident population. Since the people who attended the surgery are, in general, heterogeneously distributed for age, sex and socio-economic level, we considered them to be representative of the population of consumers. The interviews were conducted on randomly selected days of the week from November 2007 to February 2008 after the end of medical consultations. Note that we started to submit Questionnaire A and B some months after Questionnaire C, but the public awareness about opisthorchiasis was boosted several times during the entire study period as a consequence of repeated outbreaks of disease from 2007 to 2011.

All the answers to questionnaires A, B and C were recorded in an electronic database. A descriptive analysis of the answers was carried out using frequency distributions and percentages. A chi-square significance test of independence was applied to assess the association between common citizen's personal characteristics (age categorised into three classes: 15–36, 37–50, 51–88, and gender) and their freshwater fish consumption habits (question 1). The level of significance was set at p -value = 0.05. SPSS software, v. 19 (IBM, Armonk, NY, USA) was used to analyse the data.

RESULTS

Questionnaire A

We interviewed 38 fishermen (response rate = 100%; male = 38; mean age = 55; range 29–78). The results of Questionnaire A are shown in Table 1. A majority of fishermen indicated that they caught *Tinca tinca* ($n = 26$; 68%; 95% CI 0.52–0.83).

Some fishermen ($n = 17$; 45%; 95% CI 0.28–0.61) said that they cleaned ($n = 15$; 40%) and filleted ($n = 1$; 3%) the fish immediately after capture. Only one Primary Production Operator did not answer the question on how the fish were treated on capture. Cats ($n = 15$; 40%; 95% CI 0.23–0.55) were reported to roam in the berthing areas. Furthermore, some Primary Production Operators ($n = 7$; 18%; 95% CI 0.05–0.31) admitted they were aware of raw sewage discharge into the lake, a potential source of eggs of *Opisthorchis felineus* that could infect the snail first intermediate host.

Most fishermen sold fish to small and large retail stores (e.g. hypermarkets, supermarkets, discount stores, mini-markets, etc.) ($n = 35$; 92%; 95% CI 0.83–1). Some fishermen said they directly supplied small quantities of fish to the final consumer or to local fish shops ($n = 11$; 29%; 95% CI 0.13–0.44).

Questionnaire B

We interviewed 31 Post-Primary Production Operators (response rate 78%; female: $n = 12$; male: $n = 19$; mean age = 49; range 19–75). The results of Questionnaire B are shown in Table 2.

Freshwater fish meals, especially whitefish *Coregonus lavaretus* (Linnaeus) ($n = 25$; 92%; 95% CI 0.82–1.03), perch *Perca fluviatilis* Linnaeus ($n = 18$; 67%; 95% CI 0.47–0.85) and tench (*T. tinca*) ($n = 7$; 26%; 95% CI 0.08–0.43) were served by 27 of the 31 interviewed Post-Primary Production Operators.

Raw fish material came from local ($n = 25$; 93%) or regional ($n = 5$; 19%) lakes and rivers. It was often prepared by roasting ($n = 25$; 3%; 95% CI 0.82–1.03) or frying ($n = 20$; 74%; 95% CI 0.56–0.91). However, it was sometimes processed raw as 'carpaccio' ($n = 9$; 33%; 95% CI 0.14–0.52). Whitefish was the main freshwater fish species served in raw dishes and 'carpaccio' ($n = 9$; 29%). No restaurateur indicated that he or she provided raw tench meals, according to the instructions of the local health authorities.

Table 1. Specific questionnaire a section results (primary production operators-fishermen, n = 38)

Question No. 1: “What kind of fish species do you usually catch?” *			Question No. 2: “What is the species caught more frequently?”*	
Species	Absolute frequency	Percentage of total (%)	Absolute frequency	Percentage of total (%)
<i>Coregonus lavaretus</i> (Linnaeus)	37	97	31	82
<i>Tinca tinca</i> (Linnaeus)	26	68	3	8
<i>Anguilla anguilla</i> (Linnaeus)	23	61	3	8
<i>Atherina boyeri</i> Risso	21	55	5	138
<i>Micropterus salmoides</i> (Lacépède)	17	45	1	3
<i>Esox lucius</i> Linnaeus	11	29	1	3
<i>Ictalurus melas</i> (Rafinesque)	2	5	0	0
<i>Perca fluviatilis</i> Linnaeus	9	24	0	0
<i>Cyprinus carpio</i> Linnaeus	1	3	0	0
Question No. 3: “Do you handle (clean, fillet, etc.) the fish immediately after the catch?”				
Answer			Absolute frequency	Percentage of total (%)
Yes			17	45
No			21	55
Question No. 4: “Please indicate what kind of handling do you perform on fish”				
Answer			Absolute frequency	Percentage of total (%) fishermen who answered “yes” to question 3
Evisceration			15	88
Filleting			1	6
Not answer			1	6
INFORMATION ON SALE OF FISH				
Question No. 5: “Where or whom do you sell the fishes?”*				
Answer			Absolute frequency	Percentage of total (%)
Small and large retail stores			35	92
Directly small quantities of fishes to the final consumer or to local shops			11	29
OTHER INFORMATION				
Question No. 6: “Do the cats roam in the berthing area?”				
Answer			Absolute frequency	Percentage of total (%)
Yes			15	39.5
No			23	60.5
Question No. 7: “Are you aware of presence of abusive discharge of sewage into the lake?”				
Answer			Absolute frequency	Percentage of total (%)
Yes			7	18
No			31	82

* = more than one answer was admitted

Some Post-Primary Production Operators (n = 6; 19%) stated that they did not know where the fish, that they processed, came from (table 2).

Questionnaire C

A total of 53 attendees at Viterbo's Public Health Surgery agreed to be interviewed (female: n = 29, male: n = 24; mean age = 44, range 15–88). The results of Questionnaire C are shown in Table 3.

Several respondents (n = 20; 38%; 95% CI 0.24–0.51) ate freshwater fish at least once during the previous year, among whom three (15%) ate it just once during the whole year, while 12 (60%) ate it one to three times, and four (20%) answered that they consumed freshwater fish more than three times. No significant association was found between the gender of respondent ($\chi^2_1 = 0.28$; p = 0.59) or age ($\chi^2_2 = 2.62$; p = 0.26) and the consumption of freshwater fish. Roasting (50%), frying (35%) or stewing (15%) were the most frequent cooking methods of fish meals. Whitefish (50%), tench (30%), big-scale sand smelt *Atherina boyeri* Risso (15%) and black bass *Micropterus salmoides* (Lacépède) (10%) were the most commonly consumed fresh water species. Of all the people interviewed (n = 53),

including those who did not eat fish, tench were consumed by 11% (n = 6; 95% CI 0.02–0.20).

Results of common section questions

The results of the common section questions are shown in Table 4. About half of the respondents of Questionnaires A, B and C owned a pet (fishermen: n = 20; 53%, 95% CI 0.35–0.69; restaurateurs: n = 16; 52%, 95% CI 0.32–0.70; people living in the province of Viterbo: n = 27; 51%, 95% CI 0.37–0.64). Fishermen (n = 14) and restaurateurs (n = 16) owned dogs more often while residents in the province of Viterbo owned cats more frequently (n = 15).

Pets were often fed with industrial pet-food and often or occasionally with the remains of human meals (fishermen: n = 7; 35%; restaurateurs: n = 6; 38%; people living in the province of Viterbo: n = 5; 19%) or with food cooked specifically for them. Cats were often left free to roam (fishermen: n = 14, 70%; 95% CI 0.49–0.92; restaurateurs: n = 11, 69%; 95% CI 0.49–0.98; people living in the province of Viterbo: n = 19; 36%; 95% CI 0.23–0.50).

The majority of fishermen (68%) stated that they knew about human cases of opisthorchiasis. However, 68% of people living in the province of Viterbo had no knowledge about the topic. Primary Production Operators also knew

Table 2. Results of specific questionnaire B section (post-primary production operators-restaurateurs, n = 31)

Question No. 1: “Do you sell freshwater fish meals?”							
Answer	Absolute frequency			Percentage of total (%)			
Yes	27			87			
No	4			13			
Question No. 2: “Do you serve raw freshwater fish meals?”							
Answer	Absolute frequency		Percentage of total (%)		Percentage of total restaurateurs who answered “yes” to question 1		
Yes	9		29		33		
No	18		58		67		
Question No. 3: “Which of the following species of fish are served without cooking treatment and how are they prepared?”							
	Freshwater fish species			Food processing kind			
	Absolute frequency	Percentage of total (%)	Percentage of total Restaurateurs who answered “yes” to question 1(%)	Carpaccio		Marinating	
				Absolute frequency	Percentage of total restaurateurs who answered “yes” to question 2(%)	Absolute frequency	Percentage of total restaurateurs who answered “yes” to question 2(%)
<i>Coregonus lavaretus</i> (Linnaeus)	9	29	33	9	100.0	0	0
<i>Tinca tinca</i> (Linnaeus)	0	0	0	0	0	0	0
<i>Micropterus salmoides</i> (Lacépède)	2	6	7	1	11	1	11
<i>Cyprinus carpio</i> Linnaeus	1	3	4	1	11	0	0
<i>Ictalurus melas</i> (Rafinesque)	0	0	0	0	0	0	0
<i>Anguilla anguilla</i> (Linnaeus)	1	3	4	0	0	1	11
<i>Atherina boyeri</i> Risso	0	0	0	0	0	0	0
<i>Esox lucius</i> Linnaeus	2	6	7	1	11	1	11
<i>Perca fluviatilis</i> Linnaeus	1	3	4	1	11	0	0
Other	0	0	0	0	0	0	0
Question No. 4: “Where do the fish come from?”							
Answer	Absolute frequency			Percentage of total (%)			
Local rivers and lakes	25			81			
Rivers and lakes of other provinces of Lazio	5			16			
Rivers and lakes of other Italian regions	1			3			
Foreign countries	1			3			
I do not know	6			19			
Question No. 5: “Have you got special control procedures for raw fish meals in the HACCP system?”							
Answer	Absolute frequency			Percentage of total (%)			
Yes	1			3			
No	8			26			
I do not know	22			71			

more than the other respondents about the origin of human infections (fishermen: n = 10, 26%; restaurateurs: n = 2; 6%; people living in the province of Viterbo: n = 8; 15%) and were also aware of the animal species involved in the life cycle of *O. felineus* (fishermen: n = 16; 42% restaurateurs: n = 7; 23%; people living in the province of Viterbo: n = 23; 43%).

DISCUSSION

Previous studies on the life cycle of *O. felineus* have shown that tench is the only fish species parasitised in Bolsena Lake (De Liberato et al. 2011). In Italy, tench is a fish of low commercial value; nevertheless, fishermen catch it regularly. The handling of fish immediately after capture by fishermen (fish filleting and gutting) may be a practice that supports the life cycle of the parasite, since small amounts of muscle tissues or entire discarded fishes may be left on the landing area and therefore consumed by roaming cats. The suspicion that illegal wastewater discarding occurs has not been confirmed by the appropriate authorities. Anyway, it is not clear how this could influence the life cycle.

There are only a few studies on fluke's survival after wastewater treatment (Hatam-Nahavand et al. 2015), but we think that this issue is less important in our context as human beings are not the natural definitive hosts. Indeed, cat faeces sampled from around the lake were positive for opisthorchiid eggs (De Liberato et al. 2011), but there has been no suspected infection in humans other than the notified outbreaks.

However, with heavy rain the contaminated cat faeces can be washed by the water flow into the overloaded sewer system. In this case, the faeces in the wastewater can be released directly into the lake without any treatment providing a source of eggs for the snail first intermediate hosts.

Selling fish to large retail stores, such as wholesalers, facilitates their distribution over a large geographical area and can lead to further problems such as the loss of a specific labelling for tench (“to be consumed after cooking or freeze at -20 °C for one week”) which was established in Italy as an additional preventive measure after the first opisthorchiasis outbreaks occurred. In fact, the epidemiological investigation of a large foodborne outbreak in Aos-

Table 3. Results of specific questionnaire C section (residents in the province of Viterbo, n = 53)

Question No. 1: “Did you eat freshwater fish at least once in the previous year?”		Question No. 2: “How many times did you eat freshwater fish during the year?”	
Answer	Absolute frequency (percentage of total %)	Answer	Absolute frequency (percentage of total)
Yes	20 (38)	1	3 (15%)
		2–3	12 (60%)
		4–10	2 (10%)
No	33 (62)	> 10	2 (10%)
		I do not remember	1 (5%)
Question No. 3: “Where did you eat it?”*			
Answer	Absolute frequency (percentage of total respondents who answered “yes” to question 1)		
At the restaurant	10 (50%)		
At my home	9 (45%)		
At friend’s home	4 (20%)		
Outdoors	1 (5%)		
Question No. 4: “What species of fish did you eat?”*			
Answer	Absolute frequency (percentage of total respondents who answered "yes" to question 1)		
<i>Coregonus lavaretus</i> (Linnaeus)	10 (50%)		
<i>Tinca tinca</i> (Linnaeus)	6 (30%)		
<i>Atherina boyeri</i> Risso	3 (15%)		
<i>Micropterus salmoides</i> (Lacépède)	2 (10%)		
<i>Anguilla anguilla</i> (Linnaeus)	1 (5%)		
Other (or I do not know)	4 (20%)		
Question No. 5: “How was it cooked?”*			
Answer	Absolute frequency (percentage of total respondents who answered "yes" to question 1)		
Roasted	10 (50%)		
Fried	7 (35%)		
Stewed or in humid	3 (15%)		
Smoked	2 (10%)		
Other	0		

* = more than one answer was admitted

ta city (662 km to the north of Bolsena Lake) found that this mandatory information had been removed along the food chain. Fishermen selling directly to the consumers may also increase the risk of infection, since they do not have to respect the public health regulations involving labelling laid down in EU Regulation 853/2004.

In Italy, tench is not traditionally used to prepare raw fish meals. Conversely, whitefish is the most used freshwater fish species served as carpaccio (raw preparation). Even if the consumption of local raw whitefish should not be a risk for opisthorchiasis, we must consider the possibility of commercial frauds through the replacement of fish species with a greater economic value (whitefish) with others of a lesser economic value (tench), which are often caught in Bolsena Lake. This event probably occurred during the last foodborne outbreak in the Lazio Region, when the suspected contaminated food was a whitefish carpaccio contaminated with tench tissues (Lazio Region 2011).

Only one Post-Primary Production Operator followed specific control procedures for raw fish meals, included in the Hazard Analysis and Critical Control Point (HACCP) food safety system, as requested by the authorities. The lack of control procedures for raw fish meals based on the HACCP manual is non-compliant with regulations in force and proves a poor knowledge of the hazards related to raw fish food consumption (Regulation EC 852 2004, Regulation EC 853 2004).

Traceability means the ability to trace and follow food through all of the stages of production, processing and distribution and it is a mandatory rule for food business operators in the EU (Regulation EC No. 178 2002). The lack of traceability of purchased fish, which is occasionally reported, could be an important risk factor since only some lakes in Italy are currently known to be endemic for *O. felineus* (De Liberato et al. 2011).

The stated consumption of tench by the general population appears relatively high, considering that in Italy this is a fish of low commercial value. Previous studies have shown that the probability of infection is related to the quantity of freshwater fish consumed (Dang et al. 2008, Pumidonming et al. 2018). Even if the most commonly reported ways of eating freshwater fish are roasted, fried or stewed, we cannot rule out the possibility that *O. felineus* survive these treatments if cooking temperature is inadequate to kill the parasites.

The risk of inadequate application of specific cooking and freezing (-20° C for a week) treatments (FDA 2011) that are able to kill *O. felineus* in fish products is higher at home. In this study 25% of total people interviewed ate freshwater fish products at home. These were 65% among of those who eat freshwater fish. The European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) data confirmed that the category “Household/domestic kitchen” (39%) is the most commonly reported setting in foodborne diseases.

Table 4. Topic areas: pets management, knowledge of opisthorchiasis and on preventive healthcare n = 22)

Question No. 1: "Do you have a pet?"			
Absolute frequency (percentage of total)			
	Primary Production Operators	Post Primary Production Operators	Residents in the province of Viterbo
Yes	20 (53%)	16 (52%)	27 (51%)
No	18 (47%)	15 (48%)	26 (49%)
Question No. 2: "What kind of pet do you have?"			
Absolute frequency (percentage of total that have a pet)			
	Primary Production Operators	Post Primary Production Operators	Residents in the province of Viterbo
Dog	14 (70%)	9 (56%)	10 (37%)
Cat	6 (30%)	2 (13%)	15 (56%)
Dog and cat	2 (10 %)	5 (31%)	2 (7%)
Other	0	0	0
Question No. 3: "What does your pet eat?"*			
Absolute frequency (percentage of total that have a pet)			
	Primary Production Operators	Post Primary Production Operators	Residents in the province of Viterbo
We cook for it	4 (20%)	2 (13%)	4 (15%)
Often we feed it with the remains of our meals	6 (30%)	5 (31%)	2 (7%)
Sometimes we feed it with the remains of our meals	1 (5%)	1 (6%)	3 (11%)
We feed it with pet food	18(90%)	14 (88%)	25 (93%)
Question No. 4: "Do you know how <i>O. felinus</i> infects humans?"			
Absolute frequency (percentage of total)			
	Primary production Operators	Post Primary production Operators	Residents in the province of Viterbo
Eating fish	1 (3%)	0	5 (9%)
Eating raw fish or fish not well cooked	13 (34%)	7 (23%)	5 (9%)
Eating raw fish or undercooked fish only of certain fish species	10 (26%)	2 (6%)	8 (15%)
I do not know	14 (37%)	22 (71%)	35 (66%)
Question No. 5: "Do you know of any human cases of opisthorchiasis that occurred recently?"			
Absolute frequency (percentage of total)			
	Primary production Operators	Post Primary production Operators	Residents in the province of Viterbo
Yes a lot	10 (26%)	2 (7%)	7 (13%)
Yes enough	0	10 (32%)	1 (2%)
Yes a bit	16 (42%)	7 (23%)	9 (17%)
No	12 (32%)	12 (39%)	36 (68%)
Question No. 6: "Do you know what species are involved in the <i>O. felinus</i> ' life cycle?"			
Absolute frequency (percentage of total)			
	Primary production Operators	Post Primary production Operators	Residents in the province of Viterbo
Mammals (man or cat) and fishes	16 (42%)	7 (23%)	23 (43%)
Only fishes	3 (8%)	2 (7%)	12 (23%)
Birds and fishes	1 (3%)	0	0
I do not know	18 (47%)	22 (71%)	17 (32%)
Question No. 7: "Is preventive health care adequate?"			
Absolute frequency (percentage of total)			
	Primary production Operators	Post Primary production Operators	Residents in the province of Viterbo
Yes	28 (74%)	24 (77%)	19 (36%)
No	10 (26%)	7 (23%)	31 (59%)
I do not know	0	0	3 (6%)

* = more than one answer was admitted

es outbreaks reported, followed by "Restaurant and other" (EFSA/ECDC 2015). Inadequate application of proper cooking and freezing temperatures by consumers probably occurred in the first opisthorchiasis outbreak in Viterbo, which involved 18 of 32 people who attended a dinner in a private household (Armignacco et al. 2008). Nevertheless, homemade fish meals were the cause of only two of the eight confirmed outbreaks (25%) (Crotti et al. 2007, Armignacco et al. 2008, Armignacco 2010, Traverso et al. 2010), but it is possible that under-notification of individual cases of the disease might have occurred.

Kitchen waste, which was used to feed cats and dogs by all the interviewed groups, is a potential factor for the

maintenance the life cycle of *O. felinus* together with the practice of leaving the cats to roam freely.

In any case, cats belonging to families living far from the lake are supposed not to be responsible for maintaining the cycle, even if they are not fed properly. Indeed, among the general population interviewed, only one person lived in a municipality around the lake, and he did not own cats.

Knowledge of the risk of contracting opisthorchiasis, including the life cycle and the pathways of infection, was poor in the three target populations. Related to this aspect, a possible limitation of the study may be that the recruitment of people was voluntary at the public surgery (general population of the province). This kind of selection could have

caused a selection bias in the answers to question No. 4–7 (Table 4, public health awareness) since such people could show a higher attention and consciousness towards health issues. However, due to the high percentage of respondents providing insufficient information, we think this issue did not significantly impact our results.

In conclusion, our results showed hazardous behaviours and habits among the three target populations, as well as a widespread ignorance of opisthorchiasis and preventive healthcare measures. Some habits, such as tench fishing, consumption of tench and serving of tench dishes in restaurants were underrated before this study. The recent economic downturn in Italy and the change in consumer demand for food appear to have produced an increase in the use of foods/foodstuffs of low environmental impact

and price but good nutritional value (Censis 2010), including an increased consumption of tench. This, jointly with an evolution of cooking practice, can perhaps explain the appearance of the disease only in recent years, despite the likely presence of the parasite in the lakes previously.

In order to manage the risk of human opisthorchiasis, the national and local competent authorities implemented several prevention and control measures including a rigorous application of current food safety laws. All the measures implemented by these authorities appear to be effective as the last opisthorchiasis outbreak was recorded in 2011.

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