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Second outbreak of *Trichinella pseudospiralis* in Europe: clinical patterns, epidemiological investigation and identification of the etiological agent based on the western blot patterns of the patients' serum

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Abstract

Trichinellosis is a zoonotic disease due to the ingestion of raw or undercooked meat from animals infected with the larvae of nematodes belonging to the genus *Trichinella*. In January–February 2015, an outbreak of trichinellosis occurred in Genoa, Northern Italy. The epidemiological link was traced back to a dinner served at an agritourism farm on 31 December 2014, where a majority of the 52 guests had consumed the 'beef' steak tartare. The source of infection was not traced; however, it was noted that the amount of beef purchased officially for providing at the dinner did not correspond with that served, suggesting that meat of a different origin had been added to the beef to prepare the steak tartare. Clinical and laboratory data of 30 individuals out of the 52 (57.7%), of which four were hospitalized, were consistent with that of the case definition of trichinellosis. Western blot patterns of the sera from patients with confirmed trichinellosis were similar to the diagnostic pattern identified for the reference sera of *Trichinella pseudospiralis* but different from those of the control sera tested for patients infected with *Trichinella spiralis* and *Trichinella britovi*. Identification of *T. pseudospiralis* as the aetiological agent responsible for the outbreak of trichinellosis using an indirect tool represents an advancement in the epidemiological investigation of this zoonotic disease.

KEYWORDS

epidemiology, outbreak, *Trichinella pseudospiralis*, trichinellosis, western blot, wild boar

[†]Deceased

Gómez-Morales and Mazzarello contributed equally in the study

1 | INTRODUCTION

Trichinellosis is a zoonotic disease occurring in humans by the ingestion of raw or undercooked meats of swine, horses and carnivores, and the products derived from them, which have been infected with nematode larvae belonging to the genus *Trichinella* (Gottstein, Pozio, & Nöckler, 2009). These parasites are divided into 12 taxa and exhibit a cosmopolitan distribution (Pozio & Zarlenga, 2013). Four species of *Trichinella* infecting both animals and humans have been identified in the European Union (EU): *Trichinella spiralis*, *Trichinella nativa*, *Trichinella britovi* and *Trichinella pseudospiralis*. *Trichinella nativa* and *T. britovi* are known to inhabit the Arctic and subarctic regions as well as the central and southern regions of Europe, respectively. *Trichinella spiralis*, which has been historically imported from Eastern Asia, is widely distributed in areas with a high vocation for pig breeding, which include the Balkan countries (Bulgaria, Romania and Serbia), Lithuania, Poland and Spain. *Trichinella pseudospiralis* is the only species infecting both mammals and birds and is widespread across the European continent but its prevalence is very low in susceptible animals, particularly the wild boar (Pozio, 2016; Pozio et al., 2009; Pozio & Zarlenga, 2013). According to the Commission Regulation No. 2015/1375, all *Trichinella* susceptible animals put in the market and intended for human consumption in the EU should be tested for the presence of *Trichinella* larvae in the muscles (Commission Implementing Regulation (EU) 2015/1375).

In humans, the disease is characterized by a remarkable variety of signs and symptoms associated with the widespread distribution of parasites in the body. Usually, the infection is not evident at its onset and difficulties are encountered in identifying it, particularly in singular cases. However, its presence can be suspected, if two or more individuals in the same community are afflicted simultaneously. In the acute phase, the most important hallmark is eosinophilia. Clinical forms of the infection can vary from severe to moderately severe, benign, abortive and asymptomatic; death is rare (Dupouy-Camet, Kociecka, Bruschi, Bolas-Fernandez, & Pozio, 2002). From 1986 to 2009, only 42 (0.06%) deaths were documented globally out of 65,818 cases of infection (Murrell & Pozio, 2011).

The prevalence of human trichinellosis has significantly decreased in the EU during the last decade, even if it is still documented yearly (EFSA, 2018). From 1948 to 2014, 32 outbreaks of trichinellosis (involving 1,459 cases) had occurred in Italy as a result of the consumption of meat from horses imported from the Eastern European countries (1,038, 71% of cases), free-ranging or backyard pigs (177, 12% of cases) and wild boars (244, 17% of cases) (Pozio, Ludovisi, Pezzotti, Bruschi, & Gómez-Morales, 2019). *Trichinella spiralis* and *T. britovi* were identified by molecular tools as the aetiological agents responsible for the outbreaks caused by the consumption of imported meat and that from pigs or wild boars reared or hunted in Italy, respectively. *Trichinella britovi* was identified as the species infecting domestic and wild animals in Italy with maximum frequency (97.3%), whereas the presence of *T. pseudospiralis* and *T. spiralis* was documented only in eight (2.1%) and two animals (0.5%), respectively (Garbarino et al., 2017).

Impacts

- Identification of the aetiological agent of trichinellosis by an indirect tool.
- Second outbreak of *Trichinella pseudospiralis* in Europe.
- New information on signs and symptoms of *T. pseudospiralis* in humans.
- Eosinophilia as a marker of trichinellosis in *T. pseudospiralis* infections.

The present study aimed to describe the clinical and epidemiological patterns of the trichinellosis outbreak which occurred in Northern Italy in January–February 2015 and to identify the aetiological agent responsible for the outbreak at species level using an indirect method, since neither the larvae from the infected meat nor human muscle biopsies were available.

2 | MATERIALS AND METHODS

2.1 | The event

On January 24, 2015, a woman was admitted to the emergency room of the Infectious Diseases Department of the San Martino Hospital (Genoa, Liguria region, Northern Italy). The patient was experiencing abdominal pain and eosinophilic leukocytosis. Following medical examination, she was transferred to the Clinic for Infectious Diseases. In the following days, three more individuals with similar clinical manifestations were admitted to the same hospital. A thorough anamnestic investigation revealed that all four patients had dined at the same agritourism farm event and consumed the same foods on 31 December 2014 (Figure 1).

2.2 | Epidemiological and clinical investigations

On 4 February 2015, the regional public health service, warned by the Infectious Diseases Department of the San Martino Hospital of Genoa, initiated the epidemiological investigation to trace the source of infection at the agritourism farm. Due to the time lapsed (35 days) between the occurrence of the gastronomic event and the visit for investigation, no type of fresh or frozen meat was found, since the agritourism farm is closed during winter and was opened only for the dinner event of December 31. The veterinarians could confiscate a small amount of cured meat (100 g of salami and raw ham, each, and 45 g of raw bacon) left on the agritourism farm that tested negative for the presence of the nematode larvae by the digestion method (European Commission, 2015). The agritourism farm owner declared that the beef served on the event was purchased from an authorized slaughterhouse. However, the invoice indicated the purchase of only 3 kg of beef. Although

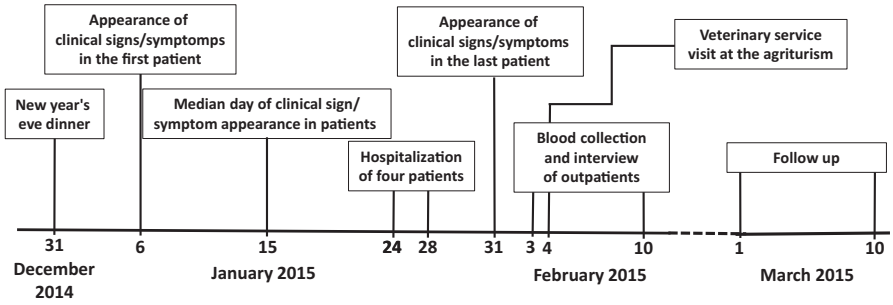

FIGURE 1 Timeline representing the trichinellosis outbreak, which occurred in Genoa, Italy, January–February 2015

50 people had claimed to have consumed several types of cured meats, the veterinary services could not recover any corresponding invoice proving their purchase.

Alongside the ongoing veterinarian investigation, all 52 guests (34 adults and 18 children) who had partaken of the dinner were also identified. The clinical history of each patient as well as the signs and symptoms developed after consumption of the dinner was documented. All adult patients who had attended the dinner were interviewed by the regional public health service to determine the types of courses served and the amount of food consumed by each individual and their children during each meal course. Additionally, the agritourism farm guests were questioned if they or their children had consumed raw meat in the days before or after the dinner of December 31. Moreover, 30 adults and 18 children underwent an outpatients' examination at the San Martino (adults) and Gaslini hospitals (children) in Genoa, Italy. Written informed consent was provided by adult patients for themselves and for their children at the hospitals. The study was approved by the Italian Ministry of Health—CCM: Azioni centrali 'Analisi epidemiologica di tre malattie infettive orfane: *Trichinella*, *Listeria*, *Echinococcus*'.

2.3 | Case definition

According to the European Centre for Disease Prevention and Control (ECDC) case definition (Commission implementing decision (EU) 2018/945), any person meeting the clinical criteria (i.e. at least three of the following six: fever, muscle pain, diarrhea, facial edema, eosinophilia, and subconjunctival, subungual and/or retinal hemorrhages) with an epidemiological link was considered as probable case, and any person meeting the clinical criteria and the laboratory criteria (antibody response specific to the *Trichinella* spp. detected using indirect immunofluorescent assay (IFA), enzyme-linked immunosorbent assay (ELISA), or western blot (WB) was considered as an confirmed case.

2.4 | Serology

Serum samples were collected from the inpatients upon admission to the hospital and outpatients at 32–33 days and 36–56 days after the event, respectively, and also at 61–69 days after the infection at follow-up. At the San Martino hospital, a commercial ELISA kit (DRG Diagnostics) was used to detect the presence of anti-*Trichinella* IgG. Refrigerated serum samples were then submitted to the National Reference Laboratory for *Trichinella*, Istituto Superiore di Sanità (ISS), Rome, Italy, for confirmatory testing. At ISS, the serum samples were tested in house using excretory/secretory antigens (ESA) through ELISA and Western blot (WB) as described previously (Gómez-Morales et al., 2008, 2012). Since the number of sera positive for the tests detected at the hospital laboratory was not consistent with that detected at ISS, the samples were further tested in house for ELISA and WB using a crude worm extract (CWE) instead of ESA (Gómez-Morales et al., 2018). As negative controls, 10 samples of sera from blood donors, which were known to be free of parasitic or other infections, were used. A total of 14 samples of human sera obtained from five subjects with confirmed *T. spiralis* and *T. britovi* infections, and four patients with confirmed *T. pseudospiralis* infections, were used as the positive control sera.

2.5 | Identification of the aetiological agent

For ethical reasons and due to the occurrence of benign clinical patterns, no muscle was biopsied from the patients. Additionally, as reported above, neither was any meat retrieved from the agritourism farm and nor larvae of *Trichinella* spp. detected in the confiscated cured meat by artificial digestion (European Commission, 2015). Therefore, in order to identify the aetiological agent responsible for the outbreak at the species level, serum samples from patients with positive ESA-ELISA and ESA-WB were tested using CWE-WB at 1/100 and 1/200 dilutions according to the method described by Gómez-Morales et al. (2018).

2.6 | Statistical analysis

Descriptive methods such as the frequency distribution, mean and/or values and range, and stratifying cases by age (i.e., adults/non-adults) were applied. Moreover, the attack rate (AR) and the exposure to specific relative risks (RR) were calculated. Box-plots were constructed to graphically compare the differences in the serological parameters between cases of raw/non-raw meat consumption and individuals with/without eosinophilia. The Wilcoxon signed-rank test was applied using EpiTools (Sergeant, 2019) to verify if these differences were statistically significant ($p < .05$ as the cut-off level).

TABLE 1 Epidemiological, clinical and laboratory features of individuals having attended the agritourism farm dinner

	N. of adults (%)	*N.* of children (%)	Total (%)
Individuals who attended the dinner	34	18	52
Gender (males)	17 (50.0)	7 (38.9)	24 (46.2)
Mean age in years	41 (range 29–60)	5.2 (range 1–13)	28.4 (range 1–60)
Individuals consuming raw meat ('beef' tartare)	31 (91.2)	3 (16.7)	34 (65.4)
Individuals consuming well cooked beef	34 (100)	9 (50.0)	43 (82.7)
Individuals consuming cured meat	34 (100)	16 (88.9)	50 (96.2)
Individuals consuming pasta stuffed with meat	34 (100)	16 (88.9)	50 (96.2)
Patients with symptoms	31 (91.2)	10 (55.6)	41 (78.8%)
Mean time (days) for the onset of symptoms post the dinner event	17 (range 6–31)	20 (range 13–32)	18 (range 6–32)
Patients with eosinophilia	31 (91.2)	6 (33.3)	37 (71.2)
Symptomatic patients with eosinophilia	29 (85.3)	4 (22.2)	33 (63.5)
Patients with positive serology[a]	33 (97.1)	3 (16.7)	36 (69.2)
Symptomatic patients with eosinophilia and negative serology	1 (2.9)	0 (0.0)	1 (1.9)
Symptomatic patients with eosinophilia and positive serology[b]	28 (82.4)	2 (11.1)	30 (57.7)
Patients with "abortive" trichinellosis	5 (14.7)	1 (5.6)	6 (11.54)

[a]Patients resulted positive by at least one of the four serological tests used: excretory/secretory antigens (ESA)-ELISA, ESA-Western blot (WB), crude worm extract (CWE)-ELISA, CWE-WB.

[b]Patients meeting the European Centre for Disease Prevention and Control definition of confirmed case of trichinellosis.

3 | RESULTS

3.1 | Epidemiological investigation

The principal results of the epidemiological investigation are presented in Table 1 and Figure 1. It was revealed that 34 (65.4%), 50 (96.1%), 43 (82.7%) and 50 (96.1%) individuals had consumed 'beef' tartare (raw meat), pasta stuffed with beef, stewed beef and cured meat, respectively. Among individuals who had consumed 'beef' tartare (raw meat), the AR was 79.4% (27/34), which slightly decreased among people who had eaten pasta stuffed with beef or cured meat (AR 60%, 30/50; RR 0.75) and among people who had consumed stewed beef (AR 62.8%, 27/43; RR 0.79) but that had not eaten 'raw meat'.

3.2 | Clinical and laboratory data

Signs and symptoms of the infection had appeared in 31 adults (91%) and 10 children (56%) after an average of 17 (range: 6–31) and 20 days (range: 13–32), respectively, from the date of the target event (Table 1, Figures 1 and 2). These included diarrhoea, abdominal pain, asthenia, fever, myalgia, night sweating, photophobia, periorbital oedema, development of rashes, conjunctivitis, cough and headaches (Table 2). The levels of eosinophils were found to be elevated in 37 patients (71%, 31 adults and six children), with a median count of 9,170 cells/μl at the time of hospital admission (inpatients, $n = 4$; outpatients, $n = 48$), 32–56 days post-infection. Of the 37 patients manifesting eosinophilia, 33 (29 adults and four children) had at least one symptom associated with *Trichinella* infection, while only 30 (28 adults and two children) demonstrated the signs and/or symptoms outlined in the ECDC case definition, AR 57.7% (30/52) (Table 1).

Upon hospital admission (either as inpatients or outpatients), the presence of anti-*Trichinella* IgG was detected in 81% of patients using the commercial kit. However, of the 52 patients, only 10 (19.2%) tested positive for *Trichinella* infection based on the results of ESA-ELISA (for screening) and ESA-WB (to confirm positive results) conducted at ISS (Table 3). Given this inconsistency between the results of the serological

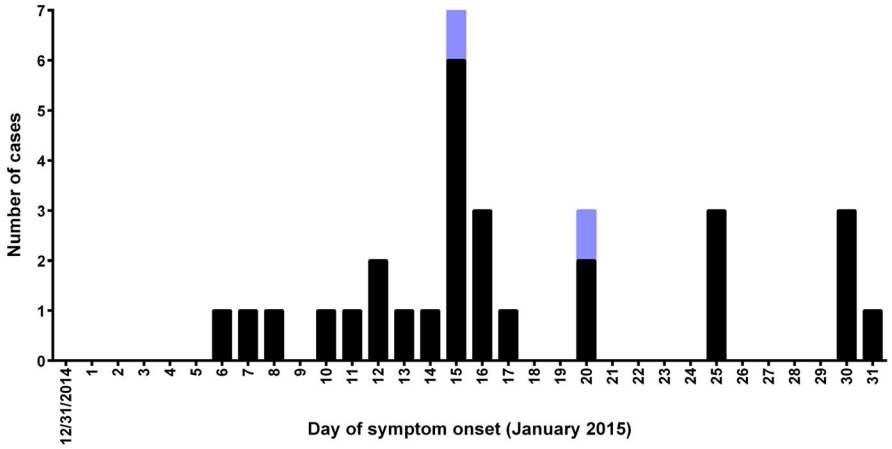

FIGURE 2 Epidemic curve. Number of patients ($n = 30$; 28 adults, black histograms; two children, blue histograms) with confirmed trichinellosis at the onset of illness in January 2015 [Colour figure can be viewed at wileyonlinelibrary.com]

tests performed at the hospital and ISS (81% vs. 19.2%), the serum samples were further tested by ELISA and WB using CWE. Thirty-six of 52 tested sera were positive by CWE-ELISA; out of 14 CWE-ELISA positive sera, 12 were positive by CWE-WB (Table 3). At follow-up (61–69 days post-infection, p.i.), from 30 people meeting the ECDC case definition for trichinellosis, serum samples of six and one persons were positive by ESA-ELISA and ESA-WB, respectively, and serum samples of 24 persons were positive by CWE-ELISA (Table 3). As shown in Figure S1, a statistically significant association was observed between the consumption of 'beef' tartare or eosinophilia with positive serology ($p < .001$, Table S1). The most important findings which had developed in these patients were diarrhoea (70.0%), eosinophilia (100.0%), fever (93.3%) and myalgia (96.7%). Periorbital oedema was documented in only four (13.3%) adults (Table 2). Less specific signs and symptoms included asthenia, night sweating, photophobia, development of rashes, conjunctivitis, cough and headache (Table 2). Leukocytosis was found in 11 (36%) patients. An increase in the levels of alanine aminotransferase (ALT) (61%) and creatine phosphokinase (CPK) (30.5%) was detected in 22 (44%) and 11 (25.5%) patients with eosinophilia, respectively, and in none with normal eosinophilic counts (Table 2). At follow-up, all patients had recovered completely (Figure 1).

Six individuals (five adults and one child) demonstrating positive serologies did not fulfil the clinical criteria outlined in the ECDC case definition, of which, five (four adults and one child) were reported to have consumed the 'beef' tartare. However, only two adults had reported myalgia and fever was the only sign reported in the child. Eosinophilia was detected in four out of the five adults. It was reported that only one person with a positive serology, who had not consumed raw meat, was asymptomatic.

TABLE 2 Clinical and laboratory features of cases with confirmed trichinellosis at the time of hospital admission as inpatients or outpatients 24–41 days post-infection

Clinical features	*N.* of adults	*N.* of children	Total (%)
Hospitalized patients	4	0	4 (13.3)
Diarrhoea	19	2	21 (70.0)
Fever >38°C	26	2	28 (93.3)
Myalgia	28	1	29 (96.7)
Periorbital oedema	4	0	4 (13.3)
Abdominal pain	19	2	21 (70.0)
Asthenia	28	2	30 (100.0)
Night sweat	13	1	14 (46.7)
Photophobia	6	1	7 (23.3)
Rash	2	1	3 (10.0)
Others (conjunctivitis, cough, headache)	4	1	5 (16.7)
Laboratory features			
Eosinophilia	28	2	30[a] (100.0)
Leukocytosis	11	0	11[b] (36.6)
Increased levels of alanine transaminase	22	0	22[c] (73.3)
Increased levels of creatine phosphokinase	11	0	11[d] (36.7)

[a]Median value, 9,170 cells/μl (range 510–23,330 cells/μl).
[b]Median value, 12,500 cells/μl (range 11,100–46,800 cells/μl).
[c]Median, 60 U/L (range 45–122 U/L).
[d]Median, 298 U/L (range 151–386 U/L).

3.3 | Treatment

Thirty-five patients with eosinophilia (29 adults and six children) were treated using albendazole (400 mg × 2 per day for 10 days, both adults and children) and prednisolone (25 mg per day for 10 days, only adults) (Dupouy-Camet et al., 2002).

3.4 | Identification of the aetiological agent

The CWE-WB patterns of the serum samples from the first blood draw of 10 patients positive for ESA-WB were similar to those of the reference samples tested using the sera of patients with confirmed *T. pseudospiralis* infection. These patterns differed from the WB patterns of the reference serum samples of patients proven to be infected with *T. spiralis* or *T. britovi* (Figure 3).

TABLE 3 Serological results of individuals having attended the agritourism farm dinner at the time of hospital admission and of people meeting case definition for trichinellosis according to the European Centre for Disease Prevention and Control (EU, 2018/945) at follow-up

	Positive/tested (%)					
	Admission 24–41 d.p.i.			Follow up 61–69 d.p.i.		
Serological test	Adults	Children	Total	Adults	Children	Total
ELISA kit[a]	25/31	1/1	26/32 (81)	n.d.	n.d.	
ESA-ELISA	11/34 (32.3)	0/18	11/52 (21.1)	6/28 (21.4)	0/2	6/30 (20)
ESA-WB	10/11	n.d.	10/11	1/6	n.d.	1/6
CWE-ELISA	33/34 (97.0)	3/18 (16.6)	36/52 (69.2)	24/28 (85.7)	0/2	24/30 (80)
CWE-WB	10/11	2/3	12/14 (85.7)	n.d.	n.d.	

Abbreviations: CWE, crude worm extract; d.p.i., days post-infection; ESA, excretory/secretory antigens; n.d., not done; WB, Western blot.
[a]DRG Diagnostics.

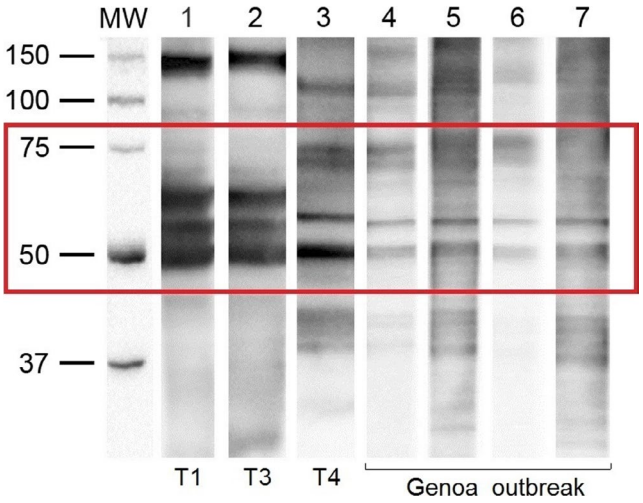

FIGURE 3 Western blot patterns of the reactivity of the crude worm extract of *Trichinella spiralis* with serum samples from those of the representative patients, who had acquired trichinellosis in the course of the outbreak, Genoa, Northern Italy, January–February 2015. Lane Mw, molecular weights in kDa; lane 1, reference serum sample at the 1:250 dilution of a patient infected with *T. spiralis* (T1); lane 2, reference serum sample at the 1:250 dilution of a patient infected with *Trichinella britovi* (T3); lane 3, reference serum sample at the 1:250 dilution of a patient infected with *Trichinella pseudospiralis* (T4); lanes 4–5, representative serum sample of patient 1 of the Genoa outbreak at the 1:200 and 1:100 dilutions, respectively; lanes 6–7, representative serum sample of patient 2 of the Genoa outbreak at 1:200 and 1:100 dilutions, respectively. The red box indicates the characteristic diagnostic pattern of recognition for each *Trichinella* species [Colour figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

During the course of trichinellosis outbreaks, species identification is a key requirement for the physicians to support the interpretation of the clinical features and laboratory diagnosis, and thus, the outcome of the infection, since certain species such as *T. spiralis* are more pathogenic than others like *T. britovi* and *T. pseudospiralis*, however for the latest only few cases are documented (Gottstein et al., 2009; Pozio, 2016). Furthermore, identification of the *Trichinella* spp. is significant in supporting the epidemiological investigations carried out by veterinary services with the following objectives: (a) to trace the source of infection; (b) prevent the spread of infected meat or derived products to other individuals; and (c) assess the risk to domestic animals susceptible to these zoonotic pathogens. Based on the Western blot patterns of the patients' sera obtained using the method proposed by Gómez-Morales et al. (2018), it was confirmed that *T. pseudospiralis* was the aetiological agent responsible for this outbreak of trichinellosis, which represents a significant advancement in the diagnosis of this zoonotic infection, particularly in the event that samples of meat infected with *Trichinella* sp. or human muscle biopsies were not available.

The EU reported that *T. pseudospiralis* was detected in animals of approximately 50% of the countries including Great Britain and Denmark (precisely in Bornholm island) (Learmount et al., 2012; Pozio, 2016). From 1999 to 2014, *T. pseudospiralis* was detected in striated muscles of two birds, one red fox (*Vulpes vulpes*), and seven wild boars in Italy (*Sus scrofa*) (Merialdi et al., 2011; Pozio, 2016). Some of these animals had been hunted in <150–300 km from Genoa, where the outbreak occurred.

Based on the outcome of the veterinary investigation and laboratory data, we suspected that meat from an unknown source, which had not been subjected to a veterinary control, was minced and mixed with the beef to prepare a tartare with enhanced flavour attributes and served it as a 'beef' dish. Based on the statements of the patients, it was established that several types of cured meats were served, including two types of salamis, of which, 100 g of the commercial leftover quantity and another homemade one served few days after the preparation according to the local tradition were tested for *Trichinella*. However, the patients were unable to remember the type of cured meat consumed by them at the dinner event. It follows that the homemade salami might have been prepared using the meat from an unknown source which could have been the source of the infection. This hypothesis was also supported by the discrepancy observed in the quantities of beef purchased and

served, wherein, only 3 kg of beef was purchased, which was used to prepare three beef dishes (beef tartare, stewed beef and pasta stuffed with beef) for 52 diners. According to the information collected from the interviews of the patients, it was noted that no less than 34 portions of beef tartare were consumed by the 52 diners. Since a portion of beef tartare weighs around 150 g, at least 5 kg of beef would have been required to prepare this dish. Furthermore, additional beef would have been required to prepare the stewed and pasta beef dishes. In this investigation, we could exclude the possibility that the consumed beef could have been a source of the infection. Moreover, based on the available data, it is known that *Trichinella* larvae have never been detected in cattle and there is only one, albeit, questionable case in which meat infected with *Trichinella* was suspected to originate from beef (Murrell, 1994).

In conclusion, given the epidemiological scenario, it is highly probable that a larger quantity of an alternative type of meat was used to supplement the low amount of purchased beef and homemade salami from the market, which was used to cater to the dinner for 52 people.

This is the second outbreak of trichinellosis caused by *T. pseudospiralis* in Europe. The previous outbreak had occurred in the Camargue region of France, about 350 km from Genoa, and involved patients who had consumed raw meat from a hunted wild boar (Ranque et al., 2000). Globally, infections of *T. pseudospiralis* have also been documented in 28 patients who had consumed pork in Kamchatka, Russia (Britov, 1997). An outbreak associated with *T. pseudospiralis* by random amplified polymorphic DNA analysis in Thailand has also been reported (Jongwutiwes et al., 1998); however, Pozio has recently suggested that this outbreak was probably caused by *Trichinella papuae* (Pozio, 2016). It follows that information on the clinical and laboratory features of *T. pseudospiralis* infections in humans is very limited since no clinical information was provided for the Kamchatka outbreak.

Determining the epidemiological link connecting the four hospitalized patients allowed the quick identification of all other 48 diners. A statistically significant association ($p < .001$) was detected among the eosinophilia level, presence of the anti-*Trichinella* IgG and consumption of the tartare served as beef, but probably containing meat from an unknown source (Figure S1). In this outbreak, the trichinellosis infection was confirmed based on the serological results of 30 (57.7%) symptomatic patients with eosinophilia (Table 1). An increase in the number of eosinophils is one of the hallmarks of trichinellosis, in which, eosinophilia is evident approximately 10 days p.i. as well as in subclinical cases (Dupouy-Camet et al., 2002). No eosinophilia was observed 34 days p.i. in two asymptomatic individuals with positive serology, who had consumed the beef tartare. These two cases could be classified as the abortive form of this infection (Table 1). The absence of eosinophilia 1-month p.i. has been documented previously in case of a woman with pancreatic failure and hepatic steatosis, presenting an infection of *T. britovi* and, in very severe cases, few days before death (Pawlowski, 1983; Romano et al., 2011). In the United States, it was reported that eosinophilia was observed in 54% of individuals out of 84 patients with confirmed trichinellosis (Wilson, Hall, Montgomery, & Jones, 2015). However, these authors did not provide information on the number of days elapsing between the onset of the infection and determination of the eosinophilic count. The incubation period (from 1 to 4 weeks) and overall clinical picture were consistent with the typical pattern observed in trichinellosis, although the duration and severity of the signs and symptoms were short and mild, respectively. In the present outbreak, diarrhoea, which is the first sign of the intestinal phase of the infection, was reported in 70% of the patients with trichinellosis. This can be considered as significantly high since, in most outbreaks, the intestinal phase often goes unnoticed in a high percentage of patients irrespective of the severity of the ensuing muscle phase. In the French outbreak of *T. pseudospiralis*, all the four patients showed diarrhoea (Ranque et al., 2000). The signs and symptoms observed during the muscle phase of the infection, which include myalgia and fever over 38°C, were reported in 93.3% and 96.7% of patients, respectively, whereas oedema and headache in only 7.7% of patients (Table 2). In the French outbreak of *T. pseudospiralis*, all the four patients showed fever over 38°C, myalgia, asthenia, whereas periorbital oedema was observed in three patients (Ranque et al., 2000). According to a review, which included reports of 5,377 global cases of trichinellosis from 1986 to 2009, myalgia (67%), oedema (55%), fever (53%), diarrhoea (27%) and headache (18%) were documented as the main signs and symptoms (Murrell & Pozio, 2011). Macular–papular rash was reported in few (8.3%) patients (Table 2), the characteristics of which was similar to those documented in cases of *T. britovi* and *T. spiralis* infections by other authors (Bruschi & Dupouy-Camet, 2014; Fichi et al., 2015; Messiaen et al., 2016). Ocular lesions as photophobia and conjunctivitis were reported in 13.8% of patients (Table 2). These clinical signs and symptoms, which manifest themselves by disturbances in the microcirculation, are common occurrences in the acute phase of the infection and are frequently reported by ophthalmologists (Dupouy-Camet et al., 2002; Pawlowski, 1983). No cardiovascular, neurological or other complications were observed. The higher prevalence of unspecific signs and symptoms in this outbreak could be associated with the concomitant occurrence of influenza, which typically affects the Italian population during winter (Vestergaard et al., 2017), and the lack of pathognomonic signs and symptoms relevant to trichinellosis. The serum sample of an asymptomatic patient, who had not consumed raw meat, had tested positive for the infection using ES ELISA. However, the low ELISA index (18.4), which was very close to that of the cut-off (11.8), suggested that it was a false positive result. Eosinophilia was observed in four serologically negative individuals upon admission to the hospital as outpatients. However, the lack of specific IgG suggests that eosinophilia probably did not occur due to trichinellosis but could be ascribed to another cause (Table 1).

The development of mild clinical patterns in 30 confirmed patients could have resulted from three concomitant causes, which are as follows: (a) low number of larvae per g in the infected meat (b) reduction of the larval burden due to mixing of the infected meat with beef, and (c) lower pathogenicity and immunogenicity

of *T. pseudospiralis* compared to that of *T. spiralis*, which is the most frequent aetiological agent causing trichinellosis globally (Bruschi & Dupouy-Camet, 2014; EFSA, 2018).

The sensitivities of serological tests used to detect anti-*Trichinella* IgG were found to be different. Of the 36 serum samples of patients that had tested positive for the infection using CWE-ELISA (Table 3), only 10 (27.7%) had also tested positive by ESA-ELISA and ESA-WB. This moderate immune response suggests that majority of the patients, including children, had probably ingested low numbers of infectious larvae.

The serological patterns observed in this study raise issues regarding the serological diagnosis of human trichinellosis. Previous studies based on the testing of well-characterized sera from patients, who had been confirmed for harbouring *Trichinella* spp. and from individuals with other parasitic or non-parasitic infections, have demonstrated that the best balance between specificity and sensitivity of serological tests can be achieved using ES antigens produced by the *T. spiralis* larvae, since these antigens are recognized by sera from hosts infected by all *Trichinella* species infecting humans (Bruschi, Gomez-Morales, & Hill, 2019; Gómez-Morales et al., 2008, 2012). However, in the present study, it was reported that the extent of production of anti-*Trichinella* IgG could have been lower than the level of IgG detection by ESA-ELISA in benign infections caused by the non-encapsulated species of *T. pseudospiralis*. The IgG response noted during this outbreak once again highlights the complexity encountered in the diagnosis of trichinellosis in the absence of a parasitological confirmation and suggests a need for the use of different groups of antigens (ESA and CWE) to achieve a diagnosis of choice in patients with mild infections, which are suspected to be those of trichinellosis.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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