

## CLINICAL AND LABORATORY ASPECTS OF A TRICHINELLOSIS OUTBREAK IN IZMIR, TURKEY

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### Summary:

Epidemiological, clinical and laboratory data were collected during an outbreak of trichinellosis, which occurred in Izmir, Turkey, between January and March 2004. The source of the infection was raw meatballs made with a mixture of uncooked beef and pork. Of 474 persons who were admitted at the Atatürk Training and Research Hospital during this period with a history of raw meatball consumption, the diagnosis of trichinellosis was confirmed for 154 (32.5 %, 87 males and 67 females; mean age 31 years, range 6-67 years). Among persons with a confirmed diagnosis, 79 % had myalgia, 77 % weakness and malaise, 63 % arthralgia, 40 % jaw pain, 68 % fever, 63 % periorbital and/or facial oedema, 49 % oedema at the trunk and limb, 42 % abdominal pain, 40 % nausea and vomiting, 28 % diarrhoea, 23 % subconjunctival haemorrhage, 25 % macular or petechial rash, 4 % subungual haemorrhage, 15 % cardiac complaints and 0.2 % neurological complaints. Nine patients (5.8 %) were hospitalised due to severe myalgia (n = 2), high fever (n = 3), neurological manifestations (n = 1), thrombophlebitis (n = 2) and palmar erythema (n = 1). Eosinophilia was present in 88 % of the confirmed cases at the admission. Elevated levels of serum creatine phosphokinase, lactic dehydrogenase and aspartate aminotransferase were detected in 72 %, 70 % and 16 % of the confirmed cases, respectively. The seroconversion occurred in most of the infected people between the 4<sup>th</sup> and 6<sup>th</sup> weeks after the infection. All of the confirmed cases were treated with mebendazole. People with severe symptoms were treated also with prednisolone (60 mg/day for three days) and those with a moderately severe clinical pattern received a non-steroid anti-inflammatory drug (naproxen sodium, 550 mg/day). All confirmed cases recovered without any clinical sequela.

**KEY WORDS :** trichinellosis, *Trichinella britovi*, outbreak, symptomatology, laboratory and epidemiological features, raw meatballs, pork, beef, Turkey.

### Résumé : ASPECTS CLINIQUES ET BIOLOGIQUES D'UNE ÉPIDÉMIE DE TRICHINELLOSE À IZMIR, EN TURQUIE

Les données épidémiologiques, cliniques et biologiques ont été collectées pendant une épidémie de trichinellose qui a eu lieu à Izmir, Turquie, entre janvier et mars 2004. L'infection a été causée par des boulettes faites d'un mélange de viandes de bœuf et de porc, consommées crues. 474 personnes ont été hospitalisées. Chez 154 d'entre elles (32,5 %; 87 hommes et 67 femmes; âge moyen de 31 ans : 6-67 ans) le diagnostic de trichinellose a été confirmé. Parmi elles : 79 % présentaient des myalgies ; 77 % un état de faiblesse et des malaises ; 63 % des arthralgies ; 40 % des douleurs de la mâchoire ; 68 % de la fièvre ; 63 % un œdème périorbitaire et/ou facial ; 49 % un œdème du tronc et des membres ; 42 % des douleurs abdominales ; 40 % des nausées et des vomissements ; 28 % une diarrée ; 23 % une hémorragie sous-conjonctivale ; 25 % un exanthème maculaire ou pétéchial ; 4 % une hémorragie sublinguale ; 15 % des troubles cardiaques ; et 0,2 % des troubles neurologiques. Neuf patients (5,8 %) ont été hospitalisés à cause d'une myalgie sévère (n = 2), d'une fièvre élevée (n = 3), de manifestations neurologiques (n = 1), d'une thrombophlébite (n = 2) et d'un érythème palmaire (n = 1). L'hyperéosinophilie était présente dans 88 % des cas confirmés à l'admission. Des taux élevés de créatine phosphokinase, de lactate déshydrogénase et d'aspartate aminotransférase sériques ont été détectés dans 72 %, 70 % et 16 % des cas, respectivement. La séroconversion est survenue chez la plupart des personnes infectées entre la 4<sup>ème</sup> et la 6<sup>ème</sup> semaine après l'infestation. Tout les cas confirmés ont été traités par mébendazole. Les patients présentant des symptômes sévères ont reçu aussi de la prednisolone (60 mg/jour pendant trois jours), et ceux présentant un tableau clinique moins sévère ont été traités avec du naproxène sodium (550 mg/jour). Tous les cas confirmés ont montré une rémission de la symptomatologie sans aucune suite clinique.

**MOTS CLÉS :** trichinellose, épidémie, *Trichinella britovi*, symptomatologie, épidémiologie, diagnostic, boulette de viande crue, porc, bœuf, Turquie.

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## INTRODUCTION

Trichinellosis, caused by nematode worms of the genus *Trichinella*, is a zoonosis with a worldwide distribution (Dupouy-Camet *et al.*, 2002). The infection is acquired through the consumption of raw or undercooked meat or meat products that harbour *Trichinella* larvae (Grove, 2000; Pozio *et al.*, 2003). Most human infections are due to *Trichinella spiralis*; while the number of infections caused by the other species is notably lower (Grove, 2000; Pozio

*et al.*, 2003). In Turkey, *Trichinella* infection is rare and had been reported in wild boars and humans (Nazli & Inal, 1987). A large outbreak of trichinellosis occurred in Izmir after the consumption of raw meatballs made of beef and pork. The incriminated food was consumed mainly, during a religious holiday in December and at the New Year's dinner. The parasite isolated from infected meatballs and from human biopsies was identified as *T. britovi* (Ozdemir *et al.*, 2005). The aim of the present study was to evaluate the clinical and laboratory features of people who were admitted at the Ataturk Training and Research Hospital (AT & RH) of Izmir, Turkey, with a history of raw meatball consumption.

## PATIENTS AND METHODS

### PATIENTS

Between January and April 2004, all people who were admitted at the Infectious Diseases and the Clinical Microbiology Departments of the AT & RH of Izmir with a history of consumption of raw meatballs, were enrolled in this study (n = 474). All persons underwent a complete physical examination. Individual data (*i.e.* age, sex, date of meatball consumption and its source) and the history of symptoms and signs were recorded. Time between the consumption of raw meatballs and the appearance of the first signs and symptoms was considered as the incubation period. During the acute phase of the outbreak, epidemiological, clinical and laboratory data were evaluated according to the algorithm suggested by Dupouy-Camet *et al.* (2002). Persons with a confirmed or a highly probable diagnosis of trichinellosis were treated with mebendazole (25 mg/kg, three times per day for two weeks). People with severe symptoms were treated also with prednisolone (60 mg/day for three days) and those with a moderately severe clinical pattern received a non-steroid anti-inflammatory drug (naproxen sodium, 550 mg/day). All of the confirmed cases were followed by clinical examination and serology up to six months after the admission, but one patient, who was followed for one year.

### LABORATORY FEATURES

Leukocyte and eosinophil counts were detected by the Cell-Dyn® 3700 test kit (Abbott, USA), according to the manufacturer's instructions. Creatine phosphokinase (CPK), lactic dehydrogenase (LDH), aspartate amino transferase (AST) and albumin values were detected in sera of the patients by the Architect C 8000™ kit (Abbott, USA), according to the manufacturer's instructions. An ELISA kit (r-biopharm AG, RIDASCREEN®

*Trichinella* IgG, Darmstadt, Germany), which uses a *T. spiralis* purified excretory/secretory antigen, was employed to detect anti-*Trichinella* antibodies in sera of enrolled people. Values > 1.1 were considered as positive, according to the manufacturer's instructions. Haematological, biochemical and serological tests were performed on all enrolled persons (n = 474) at the admission and after 15, 30 and 60 days, and at six months post infection for those with a confirmed diagnosis. Muscle biopsies (1-2 g) were taken from the deltoid muscle (n = 2) and from the gastrocnemius muscle (n = 1) of three persons four weeks after the onset of the symptoms. Each biopsy was cut into small pieces as the size of a rice grain, squashed between two microscope slides and examined for *Trichinella* larvae under a dissection microscope at 40 × magnification.

## RESULTS

During the acute phase of the outbreak of 474 persons with a history of raw meatball consumption, who were enrolled in this study, trichinellosis was confirmed for 154 (32.5 %), it was highly probable for 71 (15.07 %), probable for 60 (12.6 %), suspected for 42 (8.9 %) and very unlikely for 147 (31.0 %). On the basis of the clinical follow up and serology at six months *p.i.*, trichinellosis was further confirmed for the 154 cases, because all of them showed a seroconversion (ELISA values > 1.1), whereas all the other patients classified as "highly probable", "probable", "suspected" and "very unlikely" during the acute phase of the outbreak, were considered to be negative (ELISA values < 0.9). One *Trichinella* larva was detected in one biopsy (this patient with neurologic complications had always eaten raw meats) (Fig. 1). Among the 154 people with a confirmed diagnosis, 87 (56.5 %) were males and 67 (43.5 %) were females.



Fig. 1. – *Trichinella* larvae, detected in one case by trichinelloscopy.

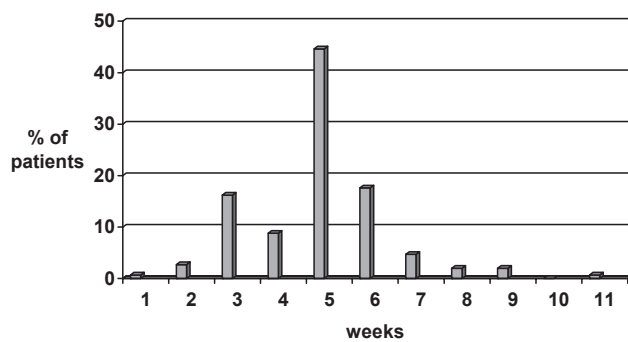


Fig. 2. – Percentage of patients (n = 148) with a confirmed diagnosis of trichinellosis showing different incubation periods (weeks).

Age range was 6-67 years (mean 31 years) with 71 % of teenagers or young adults (10-39 years).

All confirmed cases had consumed raw meatballs in the same restaurant or bought from the same market. Furthermore, 80 % of them had consumed the incriminated meatballs between December 16<sup>th</sup>, 2003 and January 1<sup>st</sup>, 2004.

The incubation period, known for 148 persons, ranged from five up to 76 days (average 31.8 days) after the consumption of raw meatballs (Fig. 2). Myalgia, weakness, malaise and fever were the most prominent sign and symptoms (Table I). The clinical pattern was classified as severe (5.8 %), moderately severe (46.1 %), benign (36.4 %), abortive (9.1 %) and asymptomatic (2.6 %). Nine people (5.8 %) were hospitalized due to severe myalgia (n = 2), uncontrolled fever (n = 3), neurological disorders (n = 1), thrombophlebitis (n = 2) and palmar erythema (n = 1).

Eosinophilia was the most prominent laboratory finding detected in 88 % of the confirmed cases at the admission and it was still present in 31 % of them two

Signs and symptoms	No. (%)	Average weeks (range)
Myalgia	122 (79)	5 (2-8)
Weakness and malaise	119 (77)	4 (2-8)
Muscle weakness	107 (70)	4 (2-8)
Fever	105 (68)	2 (1-7)
Arthralgia	97 (63)	1 (1-3)
Periorbital oedema	97 (60)	3 (1-6)
Facial oedema	93 (60)	2 (1-4)
Headache	93 (60)	2 (2-6)
Trunk and limb oedema	75 (49)	2 (1-6)
Abdominal pain	65 (42)	2 (2-4)
Jaw pain	61 (40)	2 (2-6)
Nausea and vomiting	61 (40)	1 (1-3)
Conjunctival oedema	58 (38)	1 (1-4)
Cough	55 (36)	1 (1-3)
Itching	49 (32)	1 (1-3)
Diarrhea	43 (28)	1 (1-5)
Macular or petechial rash	39 (25)	1 (1-5)
Subconjunctival haemorrhage	35 (23)	2 (1-7)
Chest pain	35 (23)	1 (1-5)
Dispnea	27 (18)	1 (1-4)
Cardiac complaints	23 (15)	2 (1-6)
Disphagia	21 (14)	1 (1-6)
Subungual haemorrhage	6 (4)	4 (1-7)
Neurological complaints	1 (0.2)	30

Table I. – Signs and symptoms of patients with confirmed trichinellosis (n = 154) and their frequency and length.

months later (Table II). High levels of CPK and LDH were detected in 71.4 % and 72 % of confirmed cases at the admission, respectively. The seroconversion occurred between the 2<sup>nd</sup> and 8<sup>th</sup> week after the infection. Anti-*Trichinella* IgG were detected in 42.2 % of confirmed cases at the admission and increased to 67.8 % and 73 % after 15<sup>th</sup> and 30<sup>th</sup> days, respectively. Sixty days after the admission, all of the confirmed cases were seropositive (Table II) and a seropositive

Laboratory tests	At admission	15 <sup>th</sup> Day	30 <sup>th</sup> Day	60 <sup>th</sup> Day
Leukocyte				
Mean (range) cells/mm <sup>3</sup>	11,211 (4,300-27,300)	9,060 (4,060-16,400)	8,340 (4,070-17,700)	7,750 (3,940-15,500)
% of persons > 10,000	57.2	34	20.5	2.7
Eosinophil				
Mean (range) per $\mu$ l	2,046 (46-17,810)	1,842 (34-6,270)	1,112 (28-5,671)	569 (11-1,768)
% of persons > 400 $\mu$ l	88	82.9	82.7	30.6
Creatine phosphokinase				
Mean (range) U/L	451 (51-3,050)	238 (56-1,508)	172 (40-1,877)	135 (43-1,325)
% of persons > 174 U/L	71.4	48	27.6	11.8
Lactic dehydrogenase				
Mean (range) U/L	603 (177-1,215)	499 (177-1,208)	413 (27-764)	375 (217-1,352)
% of persons > 450 U/L	72	61.2	26.7	16.7
Aspartate amino transferase				
Mean (range) U/L	39 (13-312)	30 (14-135)	29 (12-155)	31 (11-167)
% of persons > 37 U/L	32.2	18.8	16.7	14.3
Anti- <i>Trichinella</i> IgG				
% positive > 1.1	42.2	67.8	73	100
% borderline 0.9-1.1	7.8	7.4	9	0
% negative < 0.9	50	24.8	18	0

Table II. – Laboratory features of the confirmed cases of trichinellosis (n = 154) at the admission and 15, 30 and 60 days after.

ELISA test was also obtained for all of them, six months p.i. In four persons, the seroconversion occurred between three and 20 days before the appearance of the first signs and symptoms.

All persons with a confirmed diagnosis were treated with mebendazole. Nine people with severe symptoms received prednisolone and 71 people with a moderately severe clinical pattern received a non-steroid anti-inflammatory drug. The compliance of the patients for the treatment was good. Most people with a confirmed diagnosis of trichinellosis recovered within a mean of 8 weeks (range three weeks-12 months) after infection without any clinical sequela. Only one patient with neurologic complications showed muscle weakness for eight months and fatigue for one year.

## DISCUSSION

In Turkey, raw meatballs made of minced meat, wheat, tomato paste and hot spices is one dish at risk for meat-borne infections. Traditionally, the source of the meat was the deer, but it has been replaced by beef for economic reasons. When a greedy producer added infected pork to beef meat, a large outbreak of trichinellosis occurred in the city of Izmir. The index case was admitted at the Dokuz Eylul University Hospital Clinic of Rheumatology on January 12, 2004 with the complaints of diffuse joint and muscle pain and fever for three weeks. Trichinellosis was suspected because of facial and periorbital oedema with eosinophilia. The detailed history revealed that she and her 13 friends had eaten together raw meatballs a month previously. Although all of these persons showed a similar symptomatology, the clinical pattern was misdiagnosed as a viral infection. On the 15<sup>th</sup> of January, all of them (14 persons) had signs and symptoms and laboratory findings suggestive for trichinellosis (Ozdemir *et al.*, 2005). The local health authorities were immediately alerted. Serological tests confirmed the diagnosis of trichinellosis of the index patient and her friends. The public health authorities confiscated the minced meat suspected to be the source of the infection and *Trichinella* larvae (6.5 larvae/g) were detected. The identification of animal proteins present in the minced meat revealed that both beef and pork were present. *Trichinella* larvae were also isolated from two muscle biopsies taken from the index case (7.7 larvae/g) and from another infected person (16.7 larvae/g) (Ozdemir *et al.*, 2005). Larvae from both meatballs and human biopsies were identified as belonging to *T. britovi* (Ozdemir *et al.*, 2005).

In Turkey up to date, only small-scale outbreaks of trichinellosis were documented and the last two occurred in Bursa in 2003 (seven infected people) and in Antalya in 2004 (more than 40 infected people) (Merdivenci *et*

*al.*, 1977; Bostan *et al.*, 1999; Pozio & Zarlenga, 2005). In 1987, *Trichinella* infection was documented in domestic and wild pigs and in pork products (Nazli & Inal, 1987). Since the Muslim religion forbids the consumption of pork, this disease is very rare in the regions of the world where people obey its rules (Eisman & Einat, 1992).

*Trichinella britovi* is the etiological agent of sylvatic trichinellosis in temperate regions of Europe, Asia, North and Western Africa (Pozio *et al.*, 2005; Nezri *et al.*, in press). The main hosts are carnivores (e.g. raccoon dogs, red foxes, golden jackals, wolves, brown bears and wild boars) (Pozio, 2000). Human infections caused by this species have been documented in Algeria, France, Italy, Slovak Republic and Spain (Lopez-Hernandez *et al.*, 2000; Dubinsky *et al.*, 2001; Pozio *et al.*, 2001a; Cortes-Blanco *et al.*, 2002; Gomez-Garcia *et al.*, 2003; Herraez-Garcia *et al.*, 2003; Rodriguez-Osorio *et al.*, 2003; Gari-Toussaint *et al.*, 2004; Rodriguez *et al.*, 2004; Nezri *et al.*, in press).

The clinical pattern observed during the course of this outbreak is similar to that of other outbreaks caused by the same species *T. britovi* (Gomez-Garcia *et al.*, 2003). Sixty percent or more of persons with trichinellosis shared signs and symptoms (myalgia, weakness and malaise, muscle weakness, fever, arthralgia, periorbital oedema and headache) related to the muscle invasion, whereas only 42 % or less complained of gastrointestinal disorders (abdominal pain, nausea, vomiting and diarrhoea). The lack of signs or symptoms in more than 50 % of patients with trichinellosis in the early phase of the infection (*i.e.* in the first 7-10 days after infected meat consumption) clearly lays the problem for an early diagnosis and treatment. In addition, the gastrointestinal disorders related to a *Trichinella* infection are not pathognomonic, they are generally moderate or benign, and it is rare that they arrive at the care of the physician and, when it occurs, unlikely they are related to trichinellosis by physicians. Complications were detected in nine people and all of them were hospitalised.

The different diagnoses based on clinical and laboratory data during the acute phase of the outbreak and at the follow up, clearly stress the difficulties for the physician to make a correct diagnosis since the seroconversion can occur up to two months after the infection. At the admission (*i.e.* 15 days post infection), only 42 % of patients showed seroconversion. This is a typical pattern observed in *T. britovi*-infected people and in people infected with a low number of *T. spiralis* larvae, and it stresses the low sensitivity of the serological tests available on the market. The sensitivity can be increased at a loss of specificity by home-made tests (*e.g.* by immunofluorescence using a corpuscular antigen, or by ELISA and immunoblot using a crude soluble antigen), but they should be per-

formed by skilful personnel, because the interpretation of the results can be difficult and questionable.

Several reasons account for the difficulty to have a reliable diagnosis of trichinellosis apart from serology: 1) due to the invasive nature of a muscle biopsy, only few specimens can be collected, if any, and not always *Trichinella* larvae are found even in serologically confirmed cases; 2) the delayed appearance of clinical signs and symptoms; 3) people complaining an imaginary symptomatology mimic trichinellosis, in the course of a large outbreak, can muddle physicians; and 4) the lack of knowledge on this helminthic infection by physicians who unlikely experienced it previously. Consequently, the only objective diagnostic approach is the serology with the limit of the delayed seroconversion. These considerations explain, at least in part, why in the present outbreak, highly probable, probable and suspected cases based only on the clinical symptomatology, were not confirmed by serology.

The benzimidazole treatment was started at once when trichinellosis was suspected according to the algorithm suggested by Dupouy-Camet *et al.* (2002). The latter the treatment is prescribed, the higher the probability that the infected person will harbour viable larvae in their muscles for years, with possible persistent myalgia (Pozio *et al.*, 2003). The need of an early treatment, before the protective collagen capsule around larvae in muscles prevents the efficacy of the antihelminthic treatment, has been emphasised in other studies (Pozio *et al.*, 2001b; Dupouy-Camet *et al.*, 2002; Pozio *et al.*, 2003).

In 1982, in Lebanon, an outbreak of trichinellosis involving 2,456 people has been reported (Olaison & Ljungstrom, 1992). Two decades later, a large outbreak of trichinellosis occurred in Turkey and affected hundreds of people (Ozdemir *et al.*, 2005). In both outbreaks, raw pork (kebbeh nayyeh in the former and raw meatballs in the later), was the source of infection. These two outbreaks emphasise the need to test swine for *Trichinella* infection at the slaughtering to guarantee safe food for the consumers, especially in the view of the consumption of raw meat. Although *T. britovi* is predominantly a sylvatic species, it can infect the human being when farmers fail to rear properly domestic pigs, favouring their contact with wildlife. In this outbreak, the early diagnosis, the alert of health authorities and the identification of the infected meat allowed to stop the spread of the infection.

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