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


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 to the Atatürk Training and Research Hospital during this period with a wide 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).

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).

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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.
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 16th, 2003 and January 1st, 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 signs 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 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 2nd and 8th 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 15th and 30th days, respectively. Sixty days after the admission, all of the confirmed cases were seropositive (Table II) and a seropositive
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 15th 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) (Merdivenç 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 homemade 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 mimicked 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 anthelmintic 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 (Ozdemir et al., 2001b; Dupouy-Camet et al., 2002; Pozio et al., 2003).

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