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Re-evaluation of patients involved in a trichinellosis outbreak caused by *Trichinella britovi* 15 years after infection

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Abstract

This study re-evaluates 13 out of 48 subjects involved in a trichinellosis outbreak that occurred in Central Italy (Umbria Region) in 1988 resulting from the consumption of raw boar meat harboring *Trichinella britovi*. During the outbreak, 28 of 48 serologically positive subjects were asymptomatic, whereas 20 subjects presented one or more clinical signs including but not limited to fever, myalgia, periorbital oedema and conjunctivitis. Several patients were hospitalized with severe clinical signs requiring treatment with mebendazole and corticosteroids. Upon re-evaluation of 13 patients, none presented clinical signs; however, three still had increased CPK or LDH serum levels with some signs of electromyographic changes. In this study, enzyme immunoassays (EIA) were used to test the 13 positive sera for reactivity with *T. britovi* antigens using both excretory/secretory (E/S) antigens and a synthetic antigen composed of β-tyvelose conjugated to bovine serum albumin. Western blots (WB) were also carried out using a commercial kit. Studies using EIA with E/S antigen identified five positive sera; however, using β-tyvelose as antigen, only one positive sample was identified. Nearly all sera reacted positively with one or more *Trichinella* antigens when analyzed by WB, in particular to the 45 kDa β-tyvelose containing glycoprotein. Results indicate that *T. britovi*, though less pathogenic than other *Trichinella* species, is clearly capable of inducing sustainable sequelae.

Keywords: Late trichinellosis patients; *Trichinella britovi*; Serological tests; 45 kDa glycoprotein

1. Introduction

Trichinellosis, caused by the parasitic nematode *Trichinella*, is a worldwide zoonosis (Dupouy-Camet, 2000). The parasite can infect virtually all mammals as well as birds and reptiles, depending on the species, but humans appear to be especially prone to clinical disease (Bruschi and Murrell, 2002). While human trichinellosis has declined in Western Europe and the U.S., it is a growing public health problem in other developed and developing countries (Murrell and Pozio, 2000).

After acute infection, patients undergo a convalescent phase during which clinical manifestations result from the presence of muscle larvae (ML) in the skeletal muscles. The convalescent phase is followed by a chronic phase when larvae are still present but do

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not necessarily cause clinical signs and symptoms (Dupouy-Camet et al., 2002). The occurrence of late sequelae in trichinellosis is controversial. Some authors have coined the term ''chronic trichinellosis'' to describe''...persons who acquired *Trichinella* infection months or even years before and still have signs or symptoms'' (Dupouy-Camet et al., 2002).

Herein, we had the opportunity to re-evaluate 13 of 48 subjects involved in a trichinellosis outbreak that occurred in Central Italy (Umbria Region) near the Appennine mountains in 1988. The outbreak was caused by the consumption of sausages made with fresh, raw meat from wild boar infected with *T. britovi.* Relatives and friends of the hunters were predominantly infected (Frongillo et al., 1992). In most patients, the symptomatology was characterised by uncomplicated febrile course; however, cardiac involvement and acute abdominal pain were observed in two cases, ultimately requiring hospitalization. During the outbreak, 28 out of 48 serologically positive subjects were asymptomatic (IFA titers between 1:16 and 1:1,024), whereas 20 patients presented clinical signs such as fever (100%), myalgia (100%), periorbital edema and conjunctivitis (85%), nausea, vomiting and diarrhea (65%), and maculo-papular rash (20%). No fatalities were reported. Three patients were hospitalized because of severe clinical signs and two were treated with mebendazole and corticosteroids.

The aim of this study was to evaluate long term consequences following an acute infection of *T. britovi*, a species considered less pathogenic than *T. spiralis* (Pozio et al., 1993).

2. Materials and methods

2.1. Patients

Patient characteristics at the onset of infection are shown in Table 1. After 15 years, all patients evaluated were asymptomatic. Patients were evaluated for several laboratory findings including eosinophil and muscle enzyme (CPK and LDH) levels which are considered the most significant non-specific laboratory finding for trichinellosis (Bruschi and Murrell, 2002). Electromyography (EMG) was performed on most of patients using standard procedures.

Excretory/secretory (E/S) antigen, used in EIA tests, was collected by incubation of *T. spiralis* ML at 37 °C in MEM-Eagle (Gibco, Paisley, Scotland) for 24 h. After centrifugation, the supernatant was collected and filtered through Millipore filters (0.22 μ) concentrated to 700 μg/ml with 7.5% PEG 6000, and stored at −80 °C until used.

EIA tests, using E/S antigens or a synthetic tyvelose antigen, were carried out as previously described (Bruschi et al., 2001). Briefly, 125 ng of synthetic antigen (β-tyvelose-GalNac-bovine serum albumin disaccharide conjugate, free of α-tyvelose, and dissolved in Tris–EDTA–NaCl buffer, pH 7.3), provided by the Heska Co., Fort Collins, Co., U.S.A., or 100 μg of E/S antigen were added to each plate well, then stored at 4 °C overnight. After washing with PBS, pH 7.2, containing 0.05% Tween, and preblocking with 1% BSA, human sera (1:20 dilution in PBS) were added to plates containing

Table 1
Characteristics of patients evaluated at onset of infection

Patient initials	Sex	Clinical course	Treatment
G.G.	F	Mild	Not
C.M.	F	Mild	Not
P.Ma.	M	Moderate	Mebendazole (500 mg t.i.d) and corticosteroids
G.R.	F	Mild	Not
V.S.	M	Mild	Not
V.G.	M	Mild	Not
B.L.	F	Moderate	Not
M.A.	F	Mild	Not
F.A.	F	Mild	Not
P.Mr.	M	Mild	Not
G.K.	F	Mild	Not
V.V.	F	Mild	Not
P.Ms.	M	Moderate	Mebendazole (500 mg t.i.d) and corticosteroids