

SEASONALITY OF TRICHINELLOSIS IN PATIENTS HOSPITALIZED IN BELGRADE, SERBIA¹

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Summary:

A retrospective study of the course and outcome of trichinellosis in a series of 50 patients hospitalized at the Institute for Infectious and Tropical Diseases in Belgrade between 2001 and 2008 was performed. Clinical diagnosis of trichinellosis was based upon the patients' clinical history, symptoms and signs, and eosinophilia. The occurrence of cases showed a strong seasonality ($P < 0.0001$). The incubation period ranged between one and 33 days. The mean time between onset of symptoms and admission was nine days. Family outbreaks were the most frequent. Smoked pork products were the dominant source of infection (76 %). Fever was the most frequent clinical manifestation (90 %), followed by myalgia (80 %) and periorbital edema (76 %). 43 patients were examined serologically and 72 % of them had anti-*Trichinella* antibodies. Eosinophilia and elevated levels of serum CK and LDH were detected in 94, 50 and 56 % of the patients, respectively. All patients responded favorably to treatment with mebendazole or albendazole, but eight developed transient complications. Trichinellosis remains a major public health issue in Serbia.

KEY WORDS: trichinellosis, hospitalized patients, diagnosis, Serbia.

Résumé : VARIABILITÉ SAISONNIÈRE ET CLINIQUE DE LA TRICHINELLOSE DE PATIENTS HOSPITALISÉS À BELGRADE, EN SERBIE

Cette étude rétrospective décrit les aspects épidémiologiques, cliniques et biologiques de cas de trichinellose chez des patients hospitalisés à l'Institut des maladies contagieuses et tropicales de Belgrade, entre 2001-2008. Durant cette période, 50 patients (27 hommes et 23 femmes d'âge moyen de 39 ans) ont été hospitalisés pour une trichinellose. La survenue des cas était liée aux saisons ($P < 0,0001$). L'incubation a duré entre un et 33 jours. Le délai moyen entre l'apparition des premiers symptômes et l'admission a été de neuf jours. Il s'agissait le plus souvent d'épidémies familiales. L'origine de l'infection a été la viande de porc (88 %) ou de sanglier (8 %), généralement de la viande séchée (76 %) ou insuffisamment cuite (20 %). Les manifestations cliniques les plus fréquentes ont été la fièvre (90 %), les myalgies (80 %) et l'œdème périorbital (76 %). La sérologie, effectuée chez 43 patients, a été positive chez 72 % d'entre eux. Une hyperéosinophilie et des taux sériques élevés de CK et de LDH ont été détectés chez respectivement 94, 50 et 56 % des patients. Les patients ont été traités par mebendazole ou par albendazole. La durée moyenne de l'hospitalisation a été 15 jours. Des complications ont été observées chez huit patients. La trichinellose demeure un important problème de santé publique en Serbie.

MOTS CLÉS : trichinellose, patient, hospitalisation, diagnostic, Serbie.

Trichinellosis, a zoonotic disease caused by the nematode *Trichinella* spp. affecting various animal species, is caused by consumption of raw or undercooked contaminated meat. Eleven million people are estimated to be infected worldwide (Pozio *et al.*, 2003). Most human infections are due to *T. spiralis*,

while the number of infections caused by other species is notably lower (Pozio *et al.*, 2003). Trichinellosis caused by *T. spiralis* is frequently reported in Serbia. A recent epidemiological survey (Ljubić *et al.*, 2008) showed that between 1990 and 2006 a total of 1,995 cases of trichinellosis were recorded in Belgrade, the majority of which (1,515 cases, 75.9 %) came from 92 outbreaks.

Most patients suspected of trichinellosis are treated at the Institute for Infectious and Tropical Diseases in Belgrade; those who are asymptomatic or with a mild clinical presentation, with or without an elevated absolute eosinophil count and irrespective of the serological finding, are followed-up as out-patients. However, the clinical presentation in a number of patients requires hospitalization. The objective of this study was to analyze the epidemiological, clinical and laboratory characteristics of trichinellosis in the patients hospitalized for trichinellosis in Belgrade between 2001 and 2008.

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MATERIAL AND METHODS

Of the 371 patients with a presumed diagnosis of trichinellosis during the period from 01 January 2001 and 31 December 2008 (according to the data of the Public Health Service of Belgrade), 50 patients (13.47 %) were admitted to the Institute for Infectious and Tropical Diseases in Belgrade (Fig. 1). Decision on hospitalization was based on disease severity. As shown in Fig. 1, the proportion of hospitalized patients varied widely among the studied years, ranging from no hospitalizations in 2004 to 38.7 % patients requiring hospital treatment in 2001. In this study, we retrospectively analyzed their clinical history data.

Trichinellosis was diagnosed on the basis of epidemiological data (onset of illness following consumption of suspect meat), clinical presentation (fever, headache, nausea, diarrhea, facial or periorbital edema, myalgia) and laboratory test results [eosinophil value, serum creatine phosphokinase (CPK), lactic dehydrogenase (LDH), serology].

Serology for *Trichinella*-specific IgG antibodies was performed in 43 patients upon admission (first time point), and repeated in four patients two weeks later (second time point), using commercial tests. These included an indirect immunofluorescence assay (IIF) (INEP, Zemun, Serbia), and two enzyme immunoassays (ELISA): the RIDASCREEN® (R-Biopharm AG, Germany) and Novagnost™ (Dade Behring, Germany). All tests were performed according to the manufacturers' instructions. In the IIF, anti-*Trichinella* antibody was detected by IIF using frozen sections of *T. spiralis* larvae as antigen; samples were considered positive if fluorescence appeared at a sample dilution of 1:40 or higher. The Ridascreen ELISA was considered positive if the

sample index was higher than 1.1, while the Novagnost ELISA was considered positive if the absorbance value was higher than 15% over cut-off or >11.5 Novagnost™ Units.

Seasonal variations were analyzed by the Test for seasonality of a time series (seasonality χ^2).

RESULTS

EPIDEMIOLOGICAL DATA

Of the 50 hospitalized patients, 27 (54 %) were male and 23 (46 %) female. The mean age was 39 years (range 11-70 years). Most patients (70 %) were hospitalized in the winter (between November and February) (Fig. II). The monthly variations showed very strong seasonality (seasonality $\chi^2 = 1167.191$, $P < 0.0001$).

47 (94%) patients were from outbreaks of trichinellosis, mostly family outbreaks, and three (6 %) from sporadic infections. The source of infection was traced to pork meat products in 44 (88 %) and wild boar meat in four (8 %) patients, involving smoked pork products in 38 (76 %) [smoked pork meat (n = 27; 54 %) and sausages (n = 11; 20 %)] and insufficiently cooked meat in 10 (20 %); in two patients (4 %), the source of infection remained unknown. According to patient history data, the consumed meat and/or meat products were not subjected to mandatory veterinary inspection in 31 cases (62 %). Infected swine originated from the rural communities of Belgrade (Borča, Rakovica, Vrčin, Barajevo, Obrenovac, Lazarevac) and other regions of Serbia (Golubac, Golubinci, Ub, Leposavić, Bogatić).

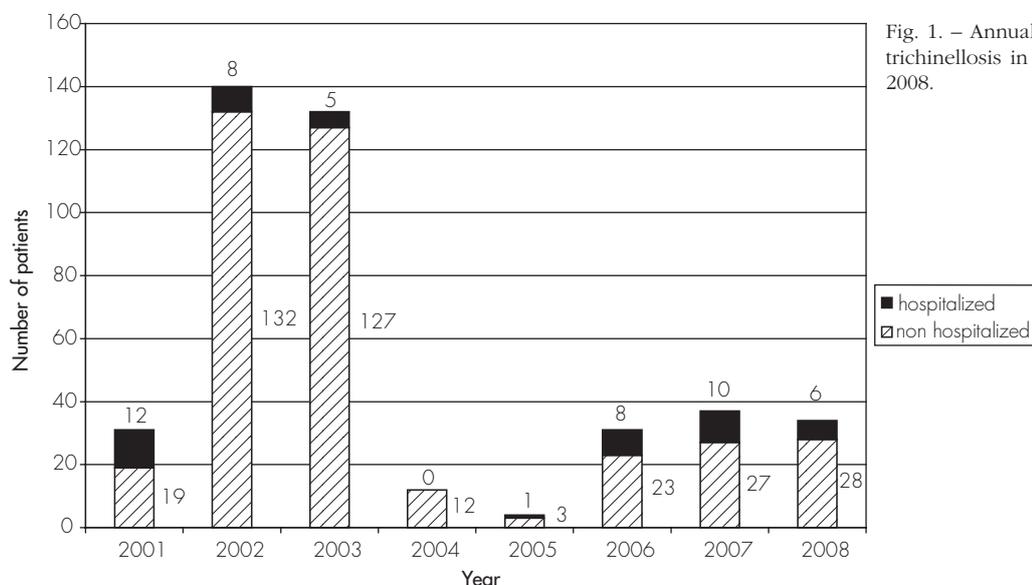


Fig. 1. – Annual distribution of patients with trichinellosis in Belgrade between 2001 and 2008.

CLINICAL DATA

In the series of 50 patients hospitalized for trichinellosis, the incubation period ranged between one and 33 days, but was most frequently between one and two weeks. The mean time between onset of symptoms and admission was nine days (range 1-22). The clinical course was variable, ranging from moderate to severe, and was probably influenced by the time lag between the onset of symptoms and admission. Clinical presentation most frequently included fever (90 %), followed by myalgia (80 %) and periorbital edema (76 %), facial edema (66 %), and diarrhea (54 %) (Table I).

In the first half of the study the patients were treated with mebendazole (n = 26; 53 %), and later with albendazole (n = 24; 47 %). In addition, 15 patients (30 %) with marked symptoms received corticosteroids. In case of serious muscle pains and high temperatures, patients also received antihistamine drugs and non-steroid anti-rheumatics. All patients had a favorable outcome following a 10-14 day course of antihelminthic treatment.

Eight hospitalized patients (16 %) developed complications, which were observed in both severe (n = 4) and moderate (n = 4) cases. Of the three patients with cardiological complications, myopericarditis with fluid in the pericardial space was observed in two in the first two weeks of diseases, associated with pain in the heart region, tachycardia and electrocardiogram abnormalities. The third patient had pericarditis with a small amount of fluid in the pericardial space and mitral valve prolapse. Two patients had respiratory complications; pneumonia due to high eosinophilia with cough, chest pain and dyspnoea appeared in the early stage of the disease, which resolved within a few days of glucocorticoid therapy. Two patients developed neuro-trichinellosis; in one, confusion, disorientation, headache and left hemiparesis appeared in the second week of the

disease. CT scan showed two small hypodense ischemic areas in the parietal regions. The other had combined neurological and cardiac complications, presenting with both right hemiparesis at day 17 post-infection, associated with diffuse and multifocal EEG changes and multiple small hypodense ischemic areas in the parietal and temporal regions on the CT scan, and acute myocarditis. One patient, who was transferred to our ICU from a local hospital, had pneumonia and acute myocardial infarction simultaneously.

The mean duration of hospitalization was 15 days (range 5-35). All confirmed cases recovered without clinical sequelae, but in patients with complications recovery lasted more than a month.

Clinical manifestation	Number (%)
Fever	45 (90)
Myalgia	40 (80)
Periorbital edema	38 (76)
Facial edema	33 (66)
Diarrhea	27 (54)
Weakness	20 (40)
Subconjunctival hemorrhage	18 (36)
Nausea	13 (26)
Abdominal pain	8 (16)
Vomiting	7 (14)
Shaking chills	6 (12)
Headache	5 (10)
Macular and petechial rash	4 (8)
Cough	4 (8)
Decreased appetite	2 (4)
Obstipation	1 (2)

Table I. – Clinical manifestations in a series of 50 patients hospitalized for trichinellosis.

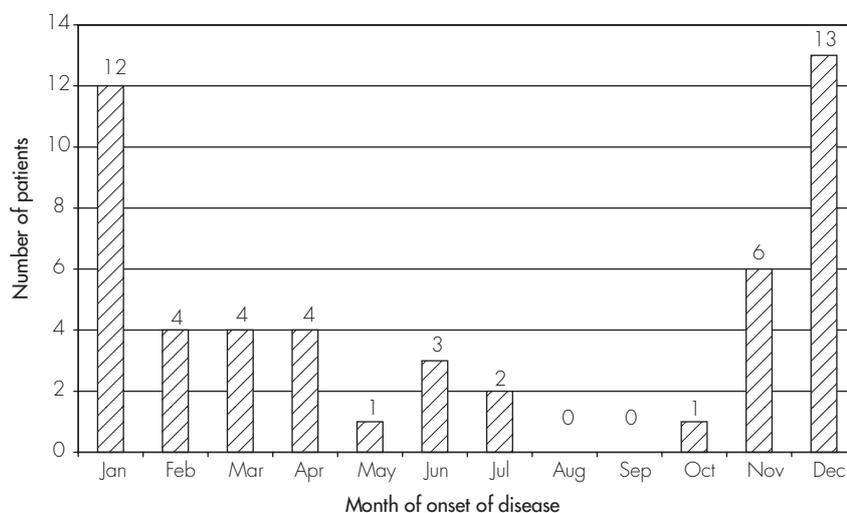


Fig. 2. – Monthly distribution of trichinellosis in a series of 50 hospitalized patients in Belgrade (2001-2008).

LABORATORY DATA

The most useful laboratory indicator was eosinophilia ($> 5.0 \times 10^9$ cells/L), which was recorded in 47 patients (94 %) at admission. Elevated levels of CPK and LDH were registered in 25 (50 %) and 28 (56 %) patients, respectively.

Trichinella-specific IgG antibodies were detected at the first time point in 27/43 (63 %) patients, of which by IFAT in 14 of the 18 (78 %) tested, and by ELISA in 13/25 (52 %). Repeated serological testing (second time point) performed in four patients, showed seroconversion in all. Thus, a total of 31 (72 %) patients were serologically positive for trichinellosis.

In two patients, the diagnosis was also confirmed by muscle biopsy (*m. gastrocnemius*).

DISCUSSION

Before 1990, swine trichinellosis in Serbia was confined to four small districts, but one third of the country is currently considered endemic for trichinellosis (Djordjević *et al.*, 2003). The conflicts associated with the dissolution of ex-Yugoslavia, resulting in socio-economic devastation of the region, are responsible for the spread of trichinellosis in the nineties (Djordjević *et al.*, 2003; Čuperlović *et al.*, 2005). Re-emerging endemic trichinellosis in Serbia has since become a serious public health issue, and reflects a high prevalence of *Trichinella* infection in domestic swine (Živojinović *et al.*, 2008). Factors contributing to the latter include failure of in-house meat inspection procedures, lack of quality assurance as well as oversight by official veterinary control (Djordjević *et al.*, 2003). As a result, 371 cases of human trichinellosis were recorded between 2001 and 2008, of which 50 (13.5 %) were hospitalized due to the severity of the disease. The wide annual differences in the proportion of hospitalized patients in our series may be a result of the time of patient arrival at our Institute (random) or may be associated with the *Trichinella* species involved, but this cannot be proved since until recently, *T. spiralis* was the only *Trichinella* species recognized in Serbia (Cvetković *et al.*, 2009).

In most cases, trichinellosis was clinically suspected based on the appearance of facial and periorbital edema, along with eosinophilia. Typical features of trichinellosis vary widely and include fever in 30-90 %, myalgia in 30-100 %, eye/facial edema in 15-100 %, and blood eosinophilia in 50-100 % of patients (Capo & Despommier, 1996). Differential diagnosis of fever and myalgia is also very wide, but appearance of periorbital or facial edema, with or without eosinophilia, help to narrow the diagnosis (Capo & Despommier, 1996). Pozio *et al.* (1993) found that the principal clinical features in

patients with *T. spiralis* were fever (60 %), myalgia (50 %), and diarrhea (40 %). The gastrointestinal disorders related to a *Trichinella* infection are not pathognomonic and are usually moderate (Turk *et al.*, 2006).

Thus, clinical diagnosis needs to be complemented with laboratory indicators, which are numerous and include eosinophilia, elevated levels of CPK and other muscle enzymes, detection of parasite-specific IgG antibodies, and anti-newborn larvae antibodies (Turk *et al.*, 2006). However, the only reliable diagnostic methods are serodiagnosis and muscle biopsy (Pozio *et al.*, 2003). Seroconversion usually occurs between the 2nd and 8th week after infection (Turk *et al.*, 2006), and the level of the parasite-specific IgG response correlates with the number of infective muscle larvae ingested (Pozio *et al.*, 1993). Wang *et al.* (1998) observed specific antibodies in only 70.2 % of patients one week after the onset of symptoms, which increased to 91, 94.3, and 100 % at two, three and four weeks, respectively. Turk *et al.* (2006) reported detection of anti-*Trichinella* IgG in 42.2 % of confirmed cases at admission, which increased to 67.8 % and 73 % after 15 and 30 days, respectively. Similarly, in our series, *Trichinella*-specific antibodies were detected in 72 % patients; the absence of specific antibodies in the others may be attributed to premature serology (since it was performed only at admission and two weeks later), as well as to the quality of the serological tests used. ELISA was shown to be more sensitive than IIF (van Knapen *et al.*, 1982), and may be used as the primary diagnostic method (Gamble *et al.*, 2004). However, the sensitivity can be increased using homemade tests; we noted better sensitivity by IIF with autochthonous antigen than by two commercial ELISA tests. Premature testing, or delayed seroconversion, may explain why in our study highly probable cases, from outbreaks, with clinical symptomatology, were not serologically confirmed. In our series, appearance within outbreaks made the diagnosis of trichinellosis easier. But in two cases of presumed trichinellosis which did not occur in outbreaks, muscle biopsy, although an invasive method, had to be performed for a definite diagnosis.

Complications of trichinellosis usually develop within the first two weeks, and appear predominantly in severe cases. Myocarditis develops in 5-20 % of all infected people. Neurological symptomatology, occurring in 3-46 % of cases, is wide because the larvae show no predilection for any particular site of the central nervous system, but may be less frequent if the infected person is treated early (Dupouy-Camet *et al.*, 2002). In our patient series, myocardial complications were the most frequent, but neurological complications had a serious course. However, all patients recovered upon treatment with mebendazole or albendazole. Albendazole and mebendazole are both active against adult worms, young migrating larvae, and larvae in muscles that have not yet encysted (Pozio *et al.*, 2003). In contrast,

mebendazole is ineffective against encapsulated muscle larvae, and this may also be the case with albendazole at this stage of the disease (Pozio *et al.*, 2001). Corticosteroids are recommended for patients with marked symptoms, but they must be administered with anthelmintics (Dupouy-Camet *et al.*, 2002). Albendazole is currently the recommended treatment of choice (CDC), and there are no trials comparing albendazole with mebendazole. In our series, in terms of clinical efficiency, no difference was observed between the subgroups of patients treated with either drug, and both treatment regimens were well tolerated.

An important observation of this study is strong seasonality of trichinellosis in Serbia during the eight studied years. As a result of specific eating and cultural habits, the phenomenon of winter seasonality of trichinellosis has been found in different areas of world, including China and Argentina, but also in the Balkan countries of Romania and Bulgaria, which both border with Serbia (Wang *et al.* 1998; Ribicich *et al.* 2005; Neghina *et al.* 2008; Kurdova-Mintcheva *et al.* 2009).

Pork-related trichinellosis is frequently reported in the Balkans, and the disease has re-emerged in Serbia, Croatia, Romania and Bulgaria in recent years (Čuperlović *et al.*, 2005). Trichinellosis in our patient series was acquired by ingestion of smoked pork products and, less frequently, of undercooked meat. The distinctive local culture may explain the seasonal occurrence, as previously done for toxoplasmosis (Bobić *et al.*, in press). Backyard pigs, which are still an important source of meat in Serbia, tend to be infected with zoonotic pathogens more often than animals raised in intensive farming systems (Klun *et al.*, 2006). Traditionally, in the Balkans, swine are slaughtered during early winter (mostly in November and December), and meat is prepared for curing, and sausages and other traditional meat products are made, sometimes before veterinary inspection of the slaughtered animals. These products are often seasoned to taste while raw, and also eaten before the necessary curing period has elapsed. In addition, Serbian culture includes celebration of the family patron saint day, of which most fall between November and January, attract friends and family from all over the country, and dishes served necessarily include different meats and meat products. This presents an opportunity for more people to contract the infection from a single source, such as improperly prepared meat, and explains the dominance of family outbreaks.

It may be concluded that trichinellosis remains a major public health issue in Serbia. The presented results emphasize the need for the the implementation of efficient prophylaxis and proper treatment in patients, as well as for more efficient veterinary control of trichinellosis in animals. The observed seasonal variations may be important when planning trichinellosis prevention programs.

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REFERENCES

- BOBIĆ B., KLUN I., NIKOLIĆ A., VUJANIĆ M., ŽIVKOVIĆ T., IVOVIĆ V. & DJURKOVIĆ-DJAKOVIĆ O. Seasonal variations in human *Toxoplasma* infection in Serbia. *Vector-Borne Zoonot Dis*, doi:10.1089/vbz.2009.0153
- CAPO V. & DESPOMMIER D.D. Clinical aspects of infection with *Trichinella* spp. *Clin. Microbiol. Rev.*, 1996, 9, 47-54.
- CENTERS FOR DISEASE CONTROL AND PREVENTION. Trichinellosis (*Trichinella spiralis*). Available at: http://www.dpd.cdc.gov/dpdx/HTML/PDF_Files/MedLetter/Trichinellosis.pdf. Accessed 1 May 2009.
- CVETKOVIĆ J., MARUCCI G., TEODOROVIĆ V., VASILEV D., VASILEV S. & SOFRONIĆ-MILOSAVLJEVIĆ Lj. Identification of *Trichinella* species in Serbia by multiplex PCR. International conference "Parasitic Zoonoses in Present Day Europe", Focus on South-East. Belgrade, Serbia, 18-20 Nov 2009. Abstract book, p. 40.
- ČUPERLOVIĆ K., DJORDJEVIĆ M. & PAVLOVIĆ S. Re-emergence of trichinellosis in southeastern Europe due to political and economic changes. *Vet. Parasitol.*, 2005, 132, 159-166.
- DJORDJEVIĆ M., BACIĆ M., PETRICEVIĆ M., ČUPERLOVIĆ K., MALAKAUSKAS A., KAPEL C.M.O. & MURRELL K.D. Social, political, and economic factors responsible for the reemergence of trichinellosis in Serbia: a case study. *J. Parasitol.*, 2003, 89, 226-231.
- DUPOUY-CAMET J., KOCIECKA W., BRUSCHI F., BOLAS-FERNANDEZ F., POZIO E. Opinion on the diagnosis and treatment of human trichinellosis. *Expert Opin. Pharmacother.*, 2002, 3, 1117-1130.
- GAMBLE H.R., POZIO E., BRUSCHI F., NOCKLER K., KAPEL C.M. & GAJADHAR A.A. International Commission on Trichinellosis: recommendations on the use of serological tests for the detection of *Trichinella* infection in animals and man. *Parasite*, 2004, 11, 3-13.
- KLUN I., DJURKOVIĆ-DJAKOVIĆ O., KATIĆ-RADIVOJEVIĆ S. & NIKOLIĆ A. Cross-sectional survey on *Toxoplasma gondii* infection in cattle, sheep and pigs in Serbia: Seroprevalence and risk factors. *Vet. Parasitol.*, 2006, 135 (2), 121-131.
- KURDOVA-MINTCHEVA R., JORDANOVA D. & IVANOVA M. Human trichinellosis in Bulgaria – Epidemiological situation and trends. *Vet. Parasitol.*, 2009, 159, 316-319.
- LJUBIĆ B., RADIVOJEVIĆ S. & RELJIĆ T. Epidemiology of zoonoses in Belgrade, 1990-2006. X Epizootology days with international participation. Tara, Serbia. 2008, Abstract book, 25-28.
- NEGHINA R., IACOBICIU I., MARINCU I., TIRNEA L., NEGHINA A.M., OLARIU R. & MOLDOVAN R. Trichinellosis, still a concern? An 18-year epidemiological surveillance of Timis county, Romania. *TMJ*, 2008, 58, 3-4.

- POZIO E., VARESE P., GOMEZ MORALES M.A., CROPPO G.P., PELLICCIA D. & BRUSCHI F. Comparison of human trichinellosis caused by *Trichinella spiralis* and by *Trichinella britovi*. *Am. J. Trop. Med. Hyg.*, 1993, 48, 568-575.
- POZIO E., SACCHINI D., SACCHI L., TAMBURRINI A. & ALBERICI F. Failure of mebendazole in the treatment of humans with *Trichinella spiralis* infection at the stage of encapsulating larvae. *Clin. Infect. Dis.*, 2001, 32, 638-642.
- POZIO E., GOMEZ MORALES M.A. & DUPOUY-CAMET J. Clinical aspects, diagnosis and treatment of trichinellosis. *Exp. Rev. Anti-Infect. Ther.*, 2003, 1, 471-482.
- RIBICICH M., GAMBLE H.R., ROSA A., BOLPE J. & FRANCO A. Trichinellosis in Argentina: an historical review. *Vet. Parasitol.*, 2005, 132, 137-142.
- TURK M., KAPTAN F., TURKER N., KORKMAZ M., EL S., OZKAYA D., URAL S., VARDAR I., ALKAN M.Z., COSKUN N.A., TURKER M. & POZIO E. Clinical and laboratory aspects of a trichinellosis outbreak in Izmir, Turkey. *Parasite*, 2006, 13, 65-70.
- VAN KNAPEN F., FRANCHIMONT J.H., VERDONK A.R., STUMPF J. & UNDEUTSCH K. Detection of specific immunoglobulins (IgG, IgM, IgA, IgE) and total IgE levels in human trichinosis by means of the enzyme-linked immunosorbent assay (ELISA). *Am. J. Trop. Med. Hyg.*, 1982, 31, 973-976.
- WANG Z.Q., CUI J., WU F., MAO F.R. & JIN X.X. Epidemiological, clinical and serological studies on Trichinellosis in Henan Province, China. *Acta Tropica*, 1998, 71, 255-268.
- ŽIVOJINOVIĆ M., DIMITRIJEVIĆ G., LAZIĆ M., PETROVIĆ M. & SOFRONIĆ-MILOSAVLJEVIĆ Ij. *Trichinella* prevalence in swine in an endemic district in Serbia: *Epidemiol. Contr.* doi:10.1016/j.vetpar.2008.10.074.

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