

TRICHINELLOSIS EPIDEMIC IN ZLATIBOR DISTRICT

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Abstract: In January 2016, the Department of Infectious Diseases in Uzice examined 111 persons with clinical and laboratory signs of trichinellosis. *Trichinella britovi* identification was performed by multiplex PCR and serodiagnosis. The patients' mayor symptoms included: myalgia (83%), weakness (82%), pain in joints (80%), fever (77%), facial edema (74%), and diarrhea (23%). Eosinophil levels of $> 500/\mu\text{l}$ were found in 98% of patients. Elevated levels of CPK were detected in 71%, LDH in 75% of patients. Three patients had cardiac complications. Treatment included mebendasole, nonsteroid anti-inflammatory drugs and corticosteroids. This outbreak indicated failures in education of all participants, from the hunters to consumers.

Keywords: trichinellosis, epidemic, *Trichinella britovi*

1. INTRODUCTION

Trichinellosis is a parasitic disease caused by nematodes of the genus *Trichinella* species. The parasite was first identified under the microscope in 1835 by Paget and Owen [1]. This is a zoonosis with a worldwide distribution [2]. The infection is caused by the ingestion of raw or undercooked meat containing encysted larvae of *Trichinella* species. Eight *Trichinella* species have been identified: *Trichinella spiralis*, *Trichinella pseudospiralis*, *Trichinella britovi*, *Trichinella nativa*, *Trichinella murrelli*, *Trichinella nelsoni*, *Trichinella papuae*, *Trichinella zimbabwensis*. Besides *Trichinella zimbabwensis* [3], all of them have been involved in human cases.

Trichinella species can infect swine, horses, wild animals (foxes, wolves, bears, skunk, raccoons, rats, and other small mammals), and humans. In wild animals, *Trichinella* infection rates vary from region to region and seems to increase in colder climates [4]. The high prevalence of trichinellosis in humans and domestic animals has been established in Bulgaria, Romania, Montenegro, Serbia and Croatia [5].

Trichinella britovi has a wide geographical range, the second most common in the human population. *Trichinella britovi* infection was documented in Europe [6], Asia, North and West Africa [7]. Pork, wild boar, and horse meat are the main sources for human infection [8].

Trichinella britovi in Serbia was first diagnosed in 2011[9]. There are also cases in the neighboring countries: Italy, Croatia, Romania, Bulgaria, Macedonia [7].

The larvae of the parasite exist in the small bowel, penetrate the mucosa, and become adults that release living larvae. The larvae migrate through the bloodstream and lymphatics and encyst within striated skeletal muscle cells. Symptoms begin with gastrointestinal irritation followed by periorbital edema, muscle pain, fever. Manifestations gradually resolve by about the third month, when the larvae have become fully encysted, although vague muscular pains and fatigue may persist for months [10].

2. PATIENTS AND METHODS

We analyzed the epidemiological, clinical and laboratory findings of a total 564 people who consumed dried meat of wild boars infected by *Trichinella britovi*. The epidemic began on the 21st of January 2016. The last patient has been viewed on February 10th, 2016.

Patients were treated and followed up at the Department of Infectious and Tropical Diseases, General Hospital in Užice, from January 2016. to March 2017. Of the total number of examined, 111 of them had clinical, biochemical and serological signs of trichinellosis.

Trichinella spp. larvae were detected in dried wild boars meat by chlorhydropeptic digestion method (0.18 larvae per gram of dry muscle and 0.87 larvae per gram of sausage). *Trichinella britovi* was identified by polymerase chain reaction analysis (PCR). Serum samples were assayed for anti-*Trichinella* IgM and IgG antibodies using the immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA) in Reference Laboratory for Trichinellosis, Institute for Application of Nuclear Energy-INEP, Belgrade and Immunological laboratories in Užice. A positive finding of *Trichinella* infestation was considered a titer greater than 1:40.

Patients were followed through the leukocyte and eosinophil counts, creatine phosphokinase (CPK), lactate dehydrogenase (LDH), alanine aminotransferase (ALT), serum albumin, immunoglobulins E-class (IgE), by standard methods that are performed in Serbia.

Patients were treated with mebendazole (20 mg/kg, three times per day 14 days, pediatric dose 500 mg three times daily for 10 days). People with severe symptoms were also treated with prednisolone (30 mg/day for 3-10 days) and a non-steroid anti-inflammatory drugs.

All patients were followed for 3 months. Patients who had clinical and laboratory indicators of infection were followed until their normalization. Serological tests were repeated on 21 patients at 12 months post infection.

3. RESULTS

From a total of 111 patients, 85 (76.6%) were males and 26 (23.4%) were females. Age range was from 2 to 84 (32.5±10). One patient was younger than 10, 12 (10.8%), were 10 – 20 years old, 17 (15.3%) were older than 65. One infected person was in the eighteenth week of pregnancy.

The incubation period was average 19.2 days (from 5 to 32 days).

The clinical course was classified as mild 57 (51.4%), moderate 45 (40.5%), and severe 9 (8.1%). Nineteen patients (17.1%) were hospitalized.

The symptoms and signs are shown in Table 1.

Table 1. The symptoms and signs

Simptoms and signs	At admission No. (%)	1st Month No. (%)	2nd Month No. (%)
Weaknes	91 (82.0)	29(26.1)	6 (5.4)
Nausea and vomiting	22 (19.8)	0	0
Diarrhea	25 (22.5)	0	0
Myalgia	92 (82.9)	13 (11.7)	3 (2.7)
Joint pain	89 (80.2)	10 (9.0)	3 (2.7)
Headache	86 (77.5)	5 (4.5)	0
Fever	85 (76.6)	0	0
Periorbital edema	85 (76.6)	0	0
Facial edema	82 (73.9)	0	0
Limb and trunc edema	73 (64.7)	0	0
Cardiac complaints	9 (8.1)	3 (2.7)	3 (2.7)

Myalgia was the dominant symptom in our patients (83%). The most patients had weakness (82%), joint pain (80%), headache (76%) too. 77% of patients had fever, in most cases (91%) was up to 38.5C. Periorbital and facial edema were dominated in the clinical course.

26% of patients had weakness after one month. Myalgia, joint pain and weakness persisted in 3-5% of patients after the second month.

Three patients had cardiac complications during the second week of the infection. Of these, two patients had myocarditis, one stroke.

Two patients (6%) had limited movement of the shoulder one year after the infection. The laboratory analysis are shown in Table 2.

Table 2. The laboratory features

Laboratory test	At admission	1st Month	2nd Month
Eosinophils			
No. (%) of persons > 500/ μ l	109 (98.2)	29 (25.7)	13 (11.7)
Mean (range) / μ l	4.347 (52-37.200)	2.007 (46-12.540)	560 (10-2.115)
Creatine phosphokinase			
No. (%) of persons > 211 IU/L	25 (71.4)	3 (8.5)	2 (5.7)
Mean (range) (IU/L)	530 (26-2.059)	310 (25-1.820)	162 (26-706)
Lactic dehydrogenase			
No. (%) of persons > 378 IU/L	83 (74.8)	13 (11.7)	2 (5.7)
Mean (range) (IU/L)	529 (112-1820)	393 (110-831)	212 (110-407)
Aspartate amino transferase			
No. (%) of persons > 34 IU/L	76 (68.5)	10 (9.0)	2 (5.7)
Mean (range) (IU/L)	69 (15-480)	52 (15-96)	44 (10-72)
Immunoglobulin E			
No.(%) of persons > 100 IU/ml	28 (25.2)	6 (5.4)	0

Laboratory analysis showed that most of the patients (98%) had eosinophilia at admission, which persisted in 12% of the patients for up to 3 months. Elevated levels of CPK were detected in 71%, LDH in 75%, AST in 68,5% of patients at admission. For total IgE, the increased levels were notified in 25.2% at admission and in 5.4% of patients after one month.

4. DISCUSSION

Trichinellosis is largely present in the Balkans, China, Russia, Argentina [7]. The number of infected people is increasing in recent decades in Serbia [11]. We were described first diagnosed cases of human infection by *Trichinella britovi* in Serbia. *Trichinella britovi* were found in wolves and red foxes in two districts, Zlatibor and Pirot. In addition, jackals, bears and raccoons were previously described as main reservoirs for *Trichinella britovi* in Serbia [9]. Our respondents consumed parasitological uninspected dried wild boars meat. Younger men were the most of the infected which corresponds to the hunting population.

The incubation period of trichinellosis varies from a few days to two months. A shorter incubation period is described in cases from Vietnam, 3-9 days [12]. In our study, the duration of incubation period was average 19.2 days.

The severity of clinical course directly correlated with the number of infective larvae ingested. The clinical pattern observed during the course of this outbreak is similar to that of other outbreaks caused by the same species *Trichinella britovi* [12-14]. About 80% of our patients had myalgia, weakness and arthralgia, more than 70% of them had facial and periorbital oedema, headache and fever, whereas only about 20 % had gastrointestinal disorders. The absence of gastrointestinal symptoms affects the later diagnosis and starting therapy.

Most of the symptoms of our patients correlate with symptoms that cause *Trichinella spiralis*, too. [15, 16]. *Trichinella spiralis* infections could be more severe than those caused by *Trichinella britovi*, and this could be due to the fact that *Trichinella britovi* females are less prolific [17].

Only 3% of our patients had complaints after two months from the beginning of infection. Clinical course of disease depends on age, sex, and general health of the infected individual as well as the infective larval load [18]. Most of our patients were younger men, while 14% of them had other diseases: diabetes mellitus, cardiovascular disease and lung cancer (one patient). These patients had a moderate clinical course of disease.

A pregnant woman was mild clinical course. She was not treated by mebendasole. After ten days, she had no complaints, biochemical tests were within the normal range. Gynecological examination has established that the trichinellosis did not affect fetal development. Described examples of fetal defects [19] caused by *Trichinella*. However, other researchers were unable to confirm transmission of trichinellosis from mother to child [20].

Eosinophilia is one of the earliest and the most characteristic laboratory findings in trichinellosis [2]. Several studies have shown that eosinophilia is correlated with the degree of myalgia [21]. The values of the number of eosinophils were increased in 98% patients in our epidemic and correlates with myalgia.

Trichinella larvae and their metabolites provoke an immune response involving many cells including eosinophils. They release enzymes such as histaminase and aryl sulfatase and damage Trichinella larvae upon activity of eosinophil cationic protein and eosinophil peroxidase. The release of histamine, serotonin, and prostaglandins increases the permeability of capillaries. This is the mechanism of occurrence of tissue edema and reduction in the level of albumin [22]. The most of our respondents had periorbital and facial edema, which is confirmed by other studies [12-14]. Edema contributes to elevated levels of IgE, in a quarter of our patients. Clinical observations suggest that IgE are responsible for the allergic manifestations typical for the clinical picture of trichinellosis [23, 24].

Migration of larvae in muscles leads to inflammation which is manifested by elevated levels of CPK and LDH. [18]. Elevated levels of the enzyme is found in more than 70% of our patients. Similar findings were presented by other authors [14, 17].

In spite of a large number of patients, there were no neurological complications. Three of them had cardiac complications. A small number of patients had myalgia for two months after the start of illness. This was surely contributed to the early initiation of therapy by mebendazole which has been emphasised in other studies [2, 25].

5. CONCLUSION

The first epidemic of trichinosis in Serbia caused by *Trichinella britovi* suggests that hunters, their families and friends can become infected when the dried meat of hunted animals is not tested for *Trichinella* before consumption. The identification of the infected meat allows to stop the spread of the infection. The early diagnosis of trichinellosis is very important for efficient treatment. In case of clinical signs such as myalgia, weakness, fever and oedema, affected patients should immediately contact a physician and, if trichinellosis is diagnosed, start treatment as soon as possible.

6. REFERENCES

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