

## SEROLOGICAL SCREENING OF PATIENTS WITH CLINICAL SUSPICION OF TRICHINELLOSIS IN BELGRADE FROM 2009 TO 2018

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### Abstract

*Introduction.* Trichinellosis is one of the most important foodborne diseases in Serbia. Most patients with suspected trichinellosis in Belgrade are referred to the Clinical Center of Serbia for diagnosis and treatment, as are unclear and complicated cases from all across Serbia.

*Materials and Methods.* A retrospective study of trichinellosis serology was carried out from 2009-2018 and included all outpatients and hospitalised patients from the Clinic for Infectious and Tropical Disease, Clinical Center of Serbia, who were serologically tested for *Trichinella* by the Parasitological Laboratory (n=1,565). *Trichinella*-specific IgG antibodies were detected in sera by a commercial ELISA test. We analysed the seroprevalence of *Trichinella*-specific IgG antibodies, antibody detection kinetics and cross-reactivity with other nematodes.

*Results and Conclusions.* The number of patients who reported for serological testing varied greatly per year and month. Most patients were tested in December and March,

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which coincides with the months with the most confirmed cases of trichinellosis. A total of 17.4% patients who were tested for trichinellosis had other parasitic infections. Altogether, 223 (14.2%) of tested patients were finally diagnosed with trichinellosis. We detected anti-*Trichinella* IgG in 68.8% (223) of patients with suspected trichinellosis on admission, which increased to 86.5%, 91.5% and 92.4% after later second, third and fourth testing, respectively. Final diagnoses of toxocariasis, strongyloidiasis, filariasis, and dirofilariasis were made for 2.4%, 0.3%, 0.3% and 0.1% of patients, respectively. Concurrent seropositivity for *Trichinella* and *Toxocara* was observed in 18.9% (7/37) of patients with clinical presentation of trichinellosis and who were also tested for toxocariasis. In 3/5 patients with imported filariasis, we found cross-reactivity with *Trichinella*. Potential cross-reactivity of this ELISA test with antibodies to the autochthonous nematode *Toxocara canis* demands the introduction of Western blot technology. Trichinellosis must be diagnosed by the combination of clinical, laboratory and epidemiological criteria.

**Key Words:** seroprevalence, IgG ELISA, human trichinellosis, cross-reactivity

## INTRODUCTION

Trichinellosis is one of the most important foodborne diseases in Serbia. Trichinellosis among swine in Serbia had a declining trend from 0.14% to 0.02% between 2001 and 2010. In same period, there were 2257 cases of human trichinellosis with 3 deaths (Sofronic-Milosavljevic et al., 2013). According to the Institute of Public Health of Serbia Dr Milan Jovanovic Batut (IOPHOS-Batut, 2018), the notification rate of trichinellosis in Serbia from 2008-2017 was stable, with annual oscillations. Compared with 2016 (2.68 cases per 100,000 inhabitants), the notification rate decreased in 2017 (0.21 cases per 100,000 inhabitants). A recurring peak occurs in winter. Outbreaks usually occurred among family members after consumption of undercooked pork meat and traditional products thereof, or wild boar and horses and their products in some years (IOPHOS-Batut, 2018). A common cause of outbreaks is consumption of unexamined meat or illegally produced/sold meat products containing *Trichinella* larvae.

The Clinic for Infectious and Tropical Diseases (CITD) at the Clinical Center of Serbia (CCS) as a University Hospital is a tertiary health care facility that treats patients with trichinellosis from Belgrade who are admitted directly to the Clinic and unclear or complicated cases who cannot be treated in other hospitals in Serbia. The Parasitological Laboratory within the CITD is the clinic diagnostic laboratory for trichinellosis. All patients with clinically suspected trichinellosis who are referred to the CCS for treatment are subjected to serological screening for trichinellosis by this laboratory.

## MATERIALS AND METHODS

A retrospective serology study of trichinellosis was carried out from January 2009 to December 2018 and included all outpatients and hospitalised patients from the Clinic for Infectious and Tropical Disease, CCS, who were serologically tested for *Trichinella*

by the Parasitological Laboratory, CCS (n=1,565). A total 1,696 sera of these patients with suspected trichinellosis were tested. Serology testing for *Trichinella*-specific IgG antibodies was performed for all 1,565 patients upon admission. Testing was repeated in 131 patients with non-reactive initial results and persistent clinical suspicion of trichinellosis. Then, 106 patients (7.7%) were tested for a second time, 20 (1.5%) for a third time and five (0.4%) for a fourth time, repeatedly using the same commercial test. Trichinellosis was diagnosed on the basis of combination of clinical presentation (fever, myalgia, diarrhoea, facial oedema, subconjunctival, subungual and retinal haemorrhages), blood eosinophilia, levels of muscle enzymes, *Trichinella*-specific antibody response and epidemiological criteria (exposure to contaminated meat or meat products), in accordance with the recent European Union (EU) *Trichinella* case definition (EU, 2018).

*Trichinella*-specific IgG antibodies were detected in sera by commercial enzyme-linked immunosorbent assay (ELISA) Novagnost® *Trichinella spiralis* IgG according to the recommendations of the manufacturer (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany). Before testing, all the sera were allowed to thaw at room temperature and they were mixed thoroughly by vortex. The sera dispensing steps (serum dilution 1+100), subsequent processing of the test (incubation 60 min, washing 3x, adding of 100 µL conjugate, incubation 30 min, wash 3x, addition of 100 µL TMB substrate, incubation 15 min, addition of 100 µL Stop solution), and measurements were performed fully automatically on the Siemens-BEP® 2000 Automatic System. This is a qualitative and semi-quantitative test. Results were reported in Novagnost® Units. Sera were considered positive if the absorbance value was higher than 15% over cut-off or >11.5 Novagnost® Units; those <8.5 were negative and, between 8.5 and 11.5 were intermediate.

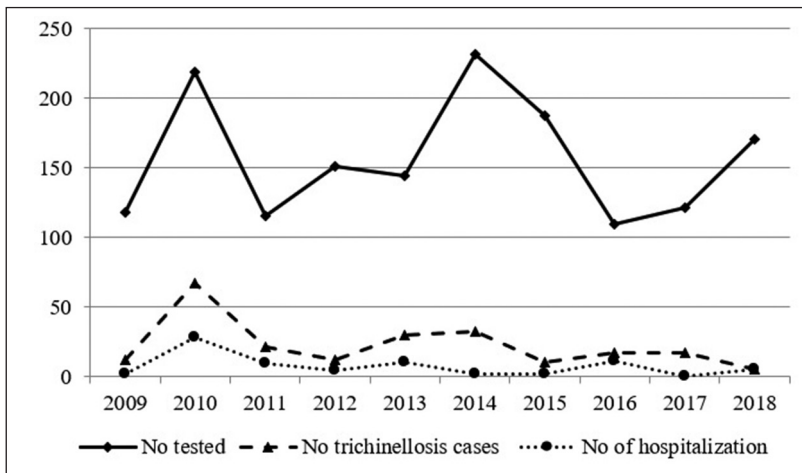
Cross-reactivity with other nematodes was also analysed. During the reporting period in this group of 1,565 patients, 601 patients were tested for *Toxocara canis*, 1,264 for intestinal helminths including 293 for *Strongyloides stercoralis* and 43 for microfilariæ, at the same time as the *Trichinella* examination was conducted.

The same type of test and protocol was used for detection of *Toxocara*-specific IgG in sera. Using the Novagnost® ELISA, sera were considered positive if the absorbance value was higher than 15% over the cut-off level or >11.5 Novagnost® Units. Three methods (direct faecal smear, formalin-ether or commercial Parasep® concentration techniques and agar plate culture) were used to examine stool specimens for diagnosis of *Strongyloides stercoralis* larvae. Blood samples were examined by the Knott method for the presence of microfilariæ.

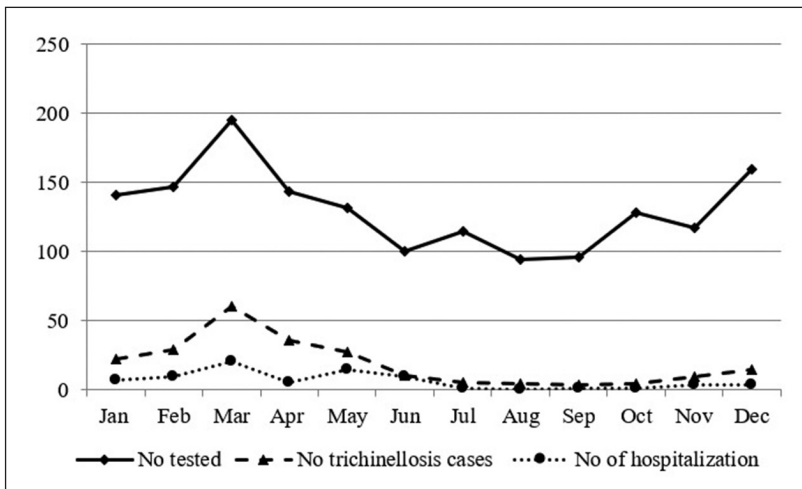
Data was obtained from laboratory protocols and the patient medical records. The statistical analysis of the data was descriptive and analytical. The statistical *t*-test, Chi-squared test and the Mann-Whitney U test were used to test significant difference between two groups. ANOVA was used for testing statistical significance for three or more groups. Distribution of frequency was analysed using the Chi-squared Goodness of Fit test.  $P < 0.05$  was considered statistically significant. The analysis was conducted in SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

Serological detection of *Trichinella*-specific IgG antibodies was performed in 1,565 patients. The mean age was  $43.1 \pm 18.1$  years (range 6-85); 737 (47.0%) were women and 828 (52.9%) men. The number of serologically tested patients varied greatly per year ( $\chi^2=111.67$ ,  $DF=9$ ,  $p<0.01$ ). Annually, from 109 (2016 y) to 231 (2014 y) sera were tested, with a mean number of  $156.6 \pm 44.01$  (Figure 1). The monthly variations in *Trichinella* test requests showed very strong seasonality ( $\chi^2=72.27$ ,  $DF=11$ ,  $p<0.01$ ). Most patients were tested in December or March (Figure 2).



**Figure 1.** Annual distribution of trichinellosis in a series of 1,565 tested patients in Belgrade from 2009 to 2018



**Figure 2.** Monthly distribution of trichinellosis in a series of 1,565 tested patients in Belgrade from 2009 to 2018

A total of 273 (17.4%) tested patients had parasitic infections. Final diagnoses of trichinellosis were ascribed to 223 (14.2%) tested patients. Final diagnoses of toxocarasis, strongyloidiasis, filariasis, and dirofilariasis were ascribed to 2.4%, 0.3%, 0.3% and 0.1% of tested patients, respectively. Separate cases of amoebiasis and trichuriasis were diagnosed in two patients (0.1%). Depending on clinical presentation and laboratory data, the other patients (n=1294; 82.7 %) were referred for further, differential diagnostic investigations (Table 1). Most of them were referred to the Allergy Clinic and the Clinic for Hematology because of the patients' unclear eosinophilia or to the Clinic of Rheumatology to differentiate causes of polymyositis.

**Table 1.** Final diagnosis of 1,565 patients who were tested for trichinellosis in Belgrade from 2009 to 2018

| Disease                     | Number | Percent (%) |
|-----------------------------|--------|-------------|
| Trichinellosis              | 223    | 14.2        |
| Toxocarasis                 | 37     | 2.4         |
| Filariasis                  | 5      | 0.3         |
| Stroglyoidiasis             | 4      | 0.3         |
| Dirofilariasis              | 2      | 0.1         |
| Amoebiasis                  | 1      | 0.1         |
| Trichuriasis                | 1      | 0.1         |
| Other diseases <sup>1</sup> | 1292   | 82.5        |

<sup>1</sup>Data about the final diagnosis are unavailable

In five patients with positive serology for *Trichinella*, trichinellosis was not confirmed. Three of these patients had imported filariasis. Microfilariae of *Loa loa* were found in the blood of two patients, while the third patient was amicrofilaraemic with Calabar swellings. One of the five patients had strong positivity for *T. canis*. These four results were interpreted as cross-reactivity with the *Trichinella* test, while in the fifth patient, the presence of other parasitic infection was not confirmed. In 12 cases, intermediate results for the *Trichinella*-specific IgG ELISA were recorded. Due to insufficient clinical and epidemiological suspicion of trichinellosis, testing was repeated. In six cases, the finding was negative, and in the other six cases, no increased antibody levels were observed. Other parasitic infections were not detected in these cases.

Among the 223 patients with clinically confirmed trichinellosis, initial detection of *Trichinella*-specific IgG antibodies was successful in 68.6% (n=153) of patients. In the second test conducted on 70 patients, seroconversion was present in 40 patients. Repeated serological testing (third time) was performed in 19 patients and showed seroconversion in 11 of these. Repeated serological testing (fourth time) was performed in two patients and showed seroconversion in both of them. Thus, a total of 206/223 (92.4%) patients were serologically positive for trichinellosis (Table 2). In the remaining 17 of the 223 patients, the testing was not repeated because they were

involved in family outbreaks and had clear clinical presentation and/or the test was not available at the time of diagnosis.

**Table 2.** Dynamics of antibody seroconversion in 223 patients with trichinellosis in Belgrade from 2009 to 2018

| No of testing | No of positive/No of tested | No of total seroconversions (%) |
|---------------|-----------------------------|---------------------------------|
| First         | 153/223                     | 153/223 (68.6)                  |
| Second        | 40/70                       | 193/223 (86.5)                  |
| Third         | 11/19                       | 204/223 (91.5)                  |
| Fourth        | 2/2                         | 206/223 (92.4)                  |

Antibodies against *T. canis* were detected in 18.9% (7/37) of tested patients, who also had trichinellosis at the same time (Table 3). Three patients with history of travel to tropical areas were tested for the presence of microfilariae, but test results were negative. Coproparasitological testing did not show the presence of strongyloidiasis (Table 3).

**Table 3.** Other parasitic infections in 223 patients with trichinellosis in Belgrade from 2009 to 2018

| Parasitic infection  | No. positive/No. tested | %    |
|----------------------|-------------------------|------|
| Toxocariasis         | 7/37                    | 18.9 |
| Filariasis           | 0/2                     | 0    |
| Intestinal parasites | 0/76                    | 0    |
| Strongyloidiasis     | 0/15                    | 0    |

The clinical course of trichinellosis was variable, ranging from inapparent infection with eosinophilia to severe disease. Of the 223 cases of trichinellosis between 2009 and 2018, 73 (32.7%) were hospitalised (Figure 1). Most hospitalised patients originated from Belgrade (78.1%) while 21.9% were from other regions of Serbia (Stara Pazova, Smederevo, Šabac etc.) or Republika Srpska, Bosnia and Hercegovina (n=3). The decision to hospitalise patients was based on disease severity. The mean hospital length of stay (LOS) was  $8.1 \pm 8.3$  days (0 to 28 days). The patients were treated with albendazole 2 x 400 mg/day for 10-15 consecutive days, along with regular administration of nonsteroidal anti-inflammatory drugs. In addition, in patients with marked symptoms, a 5-day course of corticosteroids (prednisolone) was introduced. Eleven (15.1%) hospitalised patients (4.9% of all patients with trichinellosis) developed severe or moderate but nonfatal complications. Three patients had cardiological, and two patients each had respiratory, neurological or enteral forms of complications.

Three patients with trichinellosis were primarily admitted to other Clinics (Clinics of Rheumatology, Allergy and Neurology).

In our laboratory, serological testing was conducted for 65 (89.1%) of the hospitalised patients. Due to the occasional unavailability of tests, for 8 (10.9%) patients, testing was performed in other private and public laboratories including the National Reference Laboratory (NRL) for trichinellosis. Initial diagnosis was positive in 58.5% (38/65) of these hospitalised patients, including two intermediate results in patients within family outbreaks. Testing was repeated in 22/27 patients whose initial testing was negative. The second testing was performed after  $9.6 \pm 2.2$  days (7 to 22 days), and the number of seropositive patients increased to 56/65 (86.1%). In one patient, seroconversion was recorded only in a third test (after 21 days), so the final seroconversion rate among hospitalised patients was 87.7%.

## DISCUSSION

During 2017, 224 cases of trichinellosis were recorded in EU/EEU countries with an average incidence rate of 0.03 cases per 100,000 inhabitants. The majority of confirmed cases (73.8%) in 2017 were recorded in Bulgaria (rate 0.77 cases per 100,000 population; n=55), Romania (rate 0.24; n=86) and Croatia (rate 0.51; n=37). The highest risk for this infection in the EU/EEA was consumption of undercooked meat from pigs raised under non-controlled housing conditions or hunted wild boar (ECDC, 2019).

Trichinellosis is a potentially fatal disease (Sofronic-Milosavljevic et al., 2013). Clinically manifest human trichinellosis starts with non-specific symptoms, so many other diseases should be considered during differential diagnosis (Dupuy-Camet & Bruschi, 2007; Gottstein et al., 2009). This unspecific and wide range of clinical manifestations was a main reason why so many of our patients were subjected to testing for trichinellosis. Despite the non-specific clinical manifestation of trichinellosis, new patients are usually registered in outbreaks, which makes clinical suspicion easier, but identification of initial cases can be difficult. For this reason, all recommended criteria in the EU *Trichinella* case definition should be included in analysis and carefully combined (EU, 2018).

Moreover, sporadic cases of trichinellosis, especially those with an atypical course, can be very difficult to recognise, and for this reason, trichinellosis should be excluded from various other diseases with similar clinical presentation. Non-specific symptoms were the reason three of our patients were first admitted to other specialist Clinics. Typically, trichinellosis in Serbia occurs in the form of outbreaks, most family outbreaks (Sofronic-Milosavljevic et al., 2013). The appearance of cases within outbreaks accelerated diagnosis, and was why no wider testing for other parasitic infections was performed for 223 patients with trichinellosis.

In our study, most patients presented for testing in December and March, which coincided with the months with the greatest numbers of confirmed trichinellosis cases. Traditionally in Serbia, swine are slaughtered during November and December, and undercooked meat is often consumed before veterinary inspection. Traditionally prepared meat products containing *Trichinella* larvae are usually consumed within the family circle, leading to family outbreaks. This explains the peak in testing and confirmed cases of trichinellosis in March, when meat products prepared from pigs slaughtered in November/December are ready for consumption. Our previous study showed the strong seasonality of trichinellosis in Serbia during the eight studied years (Ofori-Belić et al., 2010).

According to the case definition of trichinellosis that is suggested by the EU, a combination of a defined number of clinical criteria (fever, muscle soreness or pain, diarrhoea, facial oedema, eosinophilia, subconjunctival, subungual and retinal haemorrhages), laboratory criteria (demonstration of *Trichinella* larvae in tissue obtained by muscle biopsy or *Trichinella*-specific antibody response obtained by immunofluorescence assay (IFA), ELISA or Western blot (WB)) and epidemiological criteria (exposure to contaminated food or exposure to a common source) defines probable (clinical plus epidemiological criteria) and confirmed (clinical plus laboratory criteria) cases (EU, 2018). Although a muscle biopsy is included in the laboratory criteria of EU *Trichinella* case definition, there was no need for its use in our patients. In the previously investigated period, in two patients with presumed trichinellosis who were not associated with outbreaks, muscle biopsy, although an invasive method, had to be performed for a definite diagnosis (Ofori-Belić et al., 2010). Even though other immunological specific assays (IIF, WB) tests are commercially available in Serbia, they were not performed in our laboratory due to technical reasons. Confirmatory testing is provided by the National Reference Laboratory for trichinellosis in Serbia, which should resolve all unclear cases, but we used this only sporadically, since financing for all NRLs in Serbia has not yet been regulated. Resolving the cross-reactivity between *Toxocara* and *Trichinella* in the ELISA test we used has not yet been performed for technical reasons.

Seroconversion, i.e. production of IgG antibodies, usually occurs between 12 to 60 days after infection (Turk et al., 2006), and the level of the specific IgG response correlates with the number of infective muscle larvae ingested, *Trichinella* species and the host immunity (Pozio et al., 1993; Yang et al., 2016). The window period with a high rate of false negative results during the early stage of infection is the main disadvantage of tests detecting anti-*Trichinella* IgG (Sun et al., 2015). Repeated serological testing increased the number of serologically confirmed cases of trichinellosis. We detected anti-*Trichinella*-IgG in 68.8% of confirmed cases on admission, which increased to 86.5%, 91.5% and 92.4% after second, third and fourth testing, respectively. In hospitalised patients, seropositivity increased from 58.5% on admission to 86.1%, at second testing (after 10 days on average). There was a wide range from 7 to 22 days between the first and second tests, which depended on individual clinician's decisions. Turk et



al. (2006) reported detection of anti-*Trichinella* IgG in 42.2% of confirmed cases on admission, which increased to 67.8% and 73% after 15 and 30 days, respectively. Our recent survey between 2001 and 2008 on 50 hospitalised patients with trichinellosis showed *Trichinella*-specific IgG antibodies were detected (by the same ELISA used in the current study) in 13/25 (52%) patients on admission (Ofori-Belić et al., 2010). The mean time between onset of symptoms and admission was 9 days. Repeated serological testing two weeks later performed in four patients showed seroconversion in all (Ofori-Belić et al., 2010).

ELISA is the most commonly used approach for the detection of *Trichinella* infection in humans. However, numerous cross-reactions are possible if patients are affected by other diseases, as more often occurs in developing countries where parasitic infections are more present (Gómez-Morales et al., 2010). The sensitivity and specificity of different ELISA tests depends on the antigens used. In this study, reliable sensitivity and specificity could not be calculated because a confirmatory serological test like WB is unavailable in CCS. Sun et al. (2015) showed that in ELISA, adult worm excretory-secretory (AW ES) antigens were superior to those of the AW crude antigens and of *T. spiralis* muscle larvae (ML) ES antigens. Several studies showed that ML ES antigens have a risk of cross-reactivity with the sera of patients with other helminthiases (paragonimiasis, schistosomiasis, clonorchiasis, cysticercosis and anisakiasis) (Dea-Ayuela et al., 2001; Ciu et al., 2015; Yera et al., 2003).

According to the user manual of the commercial ELISA we used, both the sensitivity and specificity are >95%. However, the test manufacturer notes that cross-reactivity with antibodies against *T. canis* is possible. The simultaneous detection of antibodies for *Trichinella* and *Toxocara* in 18.9% of cases in our patients could be a result of cross-reaction or could indicate simultaneous infections by both autochthonous nematodes. The actual situations for individual patients would likely be clarified and more accurate if these sera were further analysed by a confirmatory WB test, which was not available to us. Using an ELISA, Gabrielli et al. (2017) reported 23.5% *Toxocara* seroprevalence, which was confirmed by confirmatory WB tests in 13.0% of the examined population in different areas of Serbia. Cross-reactivity between *Toxocara* and *Trichinella* has been documented before (Yera et al., 2003).

The basic limitation of this study is the unavailability of confirmatory WB tests for *Trichinella* and *Toxocara* in CCS. For reasons stated above, diagnosis of trichinellosis should not be established only on the basis of a single test result. Reliable diagnosis takes into consideration epidemiological data, clinical presentation and serological data.

In our hospitalised patients, the mean LOS was 8 days, which was half the LOS we determined in our previous study (Ofori-Belić et al., 2010). This could be a result of more prompt epidemic alerts and earlier patient arrival times at our Clinic. Eleven (15.1%) hospitalised patients developed complications, while in our previous study, 16% developed complications (Ofori-Belić et al., 2010). The cardiological and

neurological complications of trichinellosis that predominated in our current study are common (Dupuy-Camet & Bruschi, 2007). Gastrointestinal manifestations of trichinellosis are not pathognomonic and are usually moderate (Turk et al., 2006). However, two patients in this study developed a chronic diarrhoeal syndrome, probably due to massive ingestion of infectious muscle larvae. Albendazole was continuously administered to these patients and was well tolerated.

Our results show that trichinellosis remains a major public health problem in Serbia. Taking into account that trichinellosis not only endangers human health and causes significant economic costs, but also endangers the traditional way of life and eating habits, multidisciplinary preventive work is required to significantly reduce the threat of human trichinellosis and animal *Trichinella* infections. Strict respect of prescribed veterinary control measures and a permanent public health education program are essential.

## CONCLUSION

The diagnostic disadvantages of the ELISA used to detect *Trichinella*-specific IgG antibodies in patients means results must be interpreted carefully and in correlation with patients' clinical presentation. Particularly sensitive categories of patients are those not associated with trichinellosis outbreaks and those with history of travel to tropical areas where numerous nematode species, including loiasis, are present. Potential cross-reactivity of this ELISA test with antibodies to the autochthonous nematode *T. canis* demands the use of WB in our CCS laboratory. The availability of more types of tests, including WB, would enhance interpretation of test results, speed up trichinellosis diagnoses and reduce diagnostic errors. All diagnostically complicated cases should be analysed by the NRL. Finally, the continuous presence of human trichinellosis in Serbia requires a more repressive approach to illegal meat product marketing.

### Authors contributions

ZD designed the study, analyzed data and wrote the manuscript in consultation with all authors; NM contributed to the designed the study, performed and analyzed statistical data and wrote the manuscript in consultation with all authors; SJ, BM, IM, GS, NN, AM, AU, JM, BB analyzed data, discussed the results and contributed to the final manuscript; MK involved in planning, designing, analyzing data and supervised of this work.

### Competing interests

The authors declare that they have no competing interests.

## REFERENCES

- European Union. 2018. Commission Implementing Decision (EU) 2018/945 of 22 June 2018 on the communicable diseases and related special health issues to be covered by epidemiological surveillance as well as relevant case definitions. Trichinellosis. Official Journal of European Union, 61:49. Available at: <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L:2018:170:FULL&from=EN> Accessed 15.5.2019.
- Ciu J., Wang L., Sun G. G., Liu L. N., Zhang S. B., Liu R. D., Zhang X., Jiang P., Wang Z. Q. 2015. Characterization of a *Trichinella spiralis* 31 kDa protein and its potential application for the serodiagnosis of trichinellosis. *Acta Tropica*, 142:57–63. <http://dx.doi.org/10.1016/j.actatropica.2014.10.017>.
- Dea-Ayuela M. A., Romaris F., Ubeira F. M., Rama-Igiguez S., Martinez-Fernandez A. R., Bolas F. 2001. Possible presence of common tyvelose-containing glycans in *Trichinella* L1 larvae and embryonated eggs of several nematodes. *Parasite*, 8:S120–122. <http://dx.doi.org/10.1051/parasite/200108s2120>.
- Dupuy-Camet J., Bruschi F. 2007. Management and diagnosis of human trichinellosis. In: Dupuy-Camet J., Murrell K. D., editors. *FAO/WHO/OIE guidelines for the surveillance, management, prevention and control of trichinellosis*. Paris, 1st ed. FAO/WHO/OIE, 37–68.
- ECDC European Centre for Disease Prevention and Control. 2019. Trichinellosis. In: ECDC. *Annual epidemiological report for 2017*. Stockholm: ECDC. Available at: <https://ecdc.europa.eu/en/publications-data/trichinellosis-annual-epidemiological-report-2017>.
- Gabrielli S., Tasić-Otašević S., Ignjatović A., Fraulo M., Trenkić-Božinović M., Momčilović S., Cancrini G. 2017. Seroprevalence and risk factors for *Toxocara canis* infection in Serbia during 2015. *Foodborne Pathogens and Disease*, 14:43-49. <http://dx.doi.org/10.1089/fpd.2016.2190>.
- Gómez-Morales M. A., Ludovisi A., Amati M., Cherchi S., Pezzotti P., Pozio E. 2008. Validation of an enzyme-linked immunosorbent assay for the diagnosis of human trichinellosis. *Clinical and Vaccine Immunology*, 15:1723-1729. <http://dx.doi.org/10.1128/CVI.00257-08>.
- Gottstein B., Pozio E., Nöckler K. 2009. Epidemiology, diagnosis, treatment, and control of trichinellosis. *Clinical Microbiology Reviews*. 22:127-145. <http://dx.doi.org/10.1128/CMR.00026-08>.
- IOPHOS-Batut Institute of Public Health of Serbia Dr Milan Jovanovic *Batut*. 2018. *Annual epidemiological report for Republic of Serbia in 2017*; 49-51. Available on: <http://www.batut.org.rs/download/izvestaji/Godisnji%20izvestaji%20zarazne%20bolesti%202017.pdf>
- Ofori-Belić I., Korać M., Milošević B., Dulović O., Dakić Z., Poluga J., Brmbolić B. 2010. Seasonality of trichinellosis in patients hospitalized in Belgrade, Serbia. *Parasite*, 17:199-204. <http://dx.doi.org/10.1051/parasite/2010173199>.
- Pozio E., Varese P., Gomez Morales M. A., Croppo G. P., Pelliccia D., Bruschi F. 1993. Comparison of human trichinellosis caused by *Trichinella spiralis* and by *Trichinella britovi*. *The American Journal of Tropical Medicine and Hygiene*, 48:568-575. <http://dx.doi.org/10.4269/ajtmh.1993.48.568>.
- Sofronic-Milosavljevic Lj., Djordjevic M., Plavsic B., Grgic B. 2013. *Trichinella* infection in Serbia in the first decade of the twenty-first century. *Veterinary Parasitology*, 194:145-149. <http://dx.doi.org/10.1016/j.vetpar.2013.01.042>.
- Sun G. G., Wang Z. Q., Liu C. Y., Jiang P., Liu R. D., Wen H., Qi X., Wang L., Cui J. 2015. Early serodiagnosis of trichinellosis by ELISA using excretory–secretory antigens of *Trichinella*

- spiralis* adult worms. Parasite & Vectors, 8:484. <http://dx.doi.org/10.1186/s13071-015-1094-9>.
- 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. 2006. Clinical and laboratory aspects of a trichinellosis outbreak in Izmir, Turkey. Parasite, 13:65-70. <http://dx.doi.org/10.1051/parasite/2006131065>.
- Yang Y., Cai Y. N., Tong M. W., Vallée I., Boireau P., Liu M. Y., Cheng S. P., Song N., Xuan Y. H., Kang Y. J. 2016. Serological tools for detection of *Trichinella* infection in animals and humans. One Health, 2:25–30. <http://dx.doi.org/10.1016/j.onehlt.2015.11.005>.
- Yera H., Andiva S., Perret C., Limonne D., Boireau P., Dupouy-Camet J. 2003. Development and evaluation of a Western blot kit for diagnosis of human trichinellosis. Clinical and Diagnostic Laboratory Immunology, 10(5):793-796. <http://dx.doi.org/10.1128/CDLI.10.5.793-796.2003>.

## SEROLOŠKI SKRINING KOD PACIJENATA SA KLINIČKOM SUMNjom NA TRIHINELOZU U BEOGRADU OD 2009. DO 2018. GODINE

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### Kratak sadržaj

*Uvod.* Trihinelozu je jedna od najznačajnijih hranom prenosivih bolesti u Srbiji. Većina pacijenata iz Beograda sa sumnjom na trihinelozu se javlja u Klinički centar Srbije radi dijagnostike i lečenja, kao i nejasni i komplikovani slučajevi iz drugih krajeva Srbije.

*Materijal i metode.* Retrospektivna serološka studija sprovedena je od 2009. do 2018. godine i u nju su uključeni svi ambulantno i hospitalno lečeni pacijenti sa Klinike za infektivne i tropske bolesti u Beogradu koji su podvrgnuti serološkom testiranju na trihinelozu na Odseku za parazitologiju, Kliničkog centra Srbije (n=1.565). *Trichinella*-specifična IgG antitela su detektovana komercijalnim enzimskim imunoabsorbentnim testom. Analizirani su seroprevalenca, kinetika *Trichinella*-specifičnih IgG antitela i pojava ukrštenih reakcija sa drugim nematodama.

*Rezultati i zaključak.* Broj zahteva za serološkim testiranjem varirao je po godinama i mesecima. Najviše zahteva evidentirano je tokom decembra i marta što koincidira i sa najvećim brojem slučajeva trihineloze. Od 1.565 pacijenata testiranih na *Trichinella* kod njih 17.4% dijagnostikovane su parazitske infekcije. Trihinelozu je klinički potvrđena kod 223 (14.2%) pacijenta. Specifična anti-*Trichinella* IgG antitela detektovana su na prijemu kod 68.8% od 223 pacijenta sa trihinelozom, što se povećavalo nakon drugog (86.5%), trećeg (91.5%) i četvrtog testiranja (92.4%). Završnu dijagnozu toksokarijaze imalo je 2.4% pacijenata, strongiloidijaze i filarijaze po 0.3% i dirofilarijaze 0.1%

pacijenata. Istovremena seropozitivnost na *Trichinella* i *Toxocara* nađena je kod 18.9% (7/37) pacijenata. Kod 3/5 pacijenata sa importovanom filarijazom nađena je unakrsna pozitivnost sa *Tribinella*. Verovatna ukrštena reaktivnost sa *Toxocara canis*, autohtonom nematodom zahteva uvođenje Western blota. Dijagnostiku trihineloze trebalo bi bazirati na kombinaciji kliničkih, laboratorijskih i epidemioloških kriterijuma.

**Ključne reči:** seroprevalenca, IgG ELISA, trihineloza ljudi, ukrštena reakcija