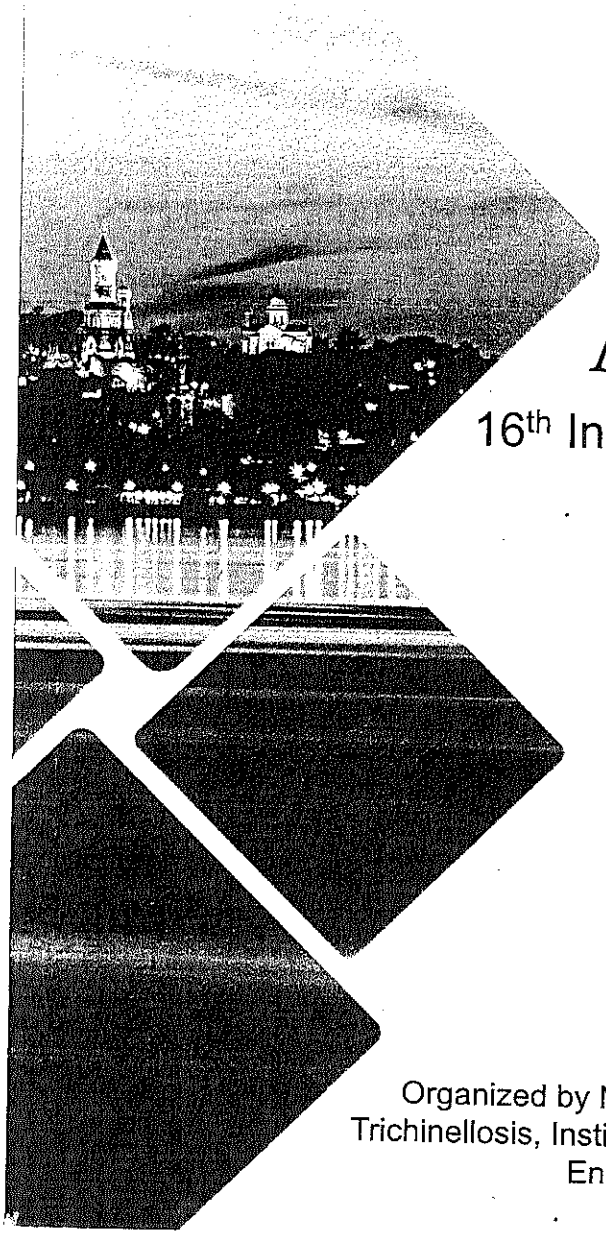
INTERNATIONAL COMMISSION
ON TRICHINELLOSIS



Program and Abstract Book

16th International Conference on Trichinellosis

August 30th - September 1st, 2023
Belgrade, Serbia

ICT 16

Organized by National Reference Laboratory for Trichinellosis, Institute for the Application of Nuclear Energy-INEP, University of Belgrade

Imprint

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Welcome Addre

Sofronic-Milosavlj
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In Serbia, infection
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Friday, 1st September 2023

10:30-11:05	**Session V: Detection, Legislation and Control (Block II, Flash poster presentations)** Chairs: Gianluca Marucci (Italy), Vasilev Saša (Serbia)
10:30-10:35	**P30. Scandrett B, Konecsni KA.** Assessment of proficiency testing samples for digestion assay using freeze-tolerant sylvatic *Trichinella* spp. with low infectivity for domestic swine.
10:35-10:40	**P31. Clara Bessi, Fernando A Fariña, Silvio J Krivokapich, Graciana Gatti, Mariano E Ercole, Francisco Montalvo, Marcelo Acerbo, Mabel M Ribicich, Mariana I Pasqualetti** Early detection of *Trichinella* infection through real-time PCR analysis in experimentally infected pigs and wild boars
10:40-10:45	**P32. Gastón Moré, Hannah Pischon, Sophie Merz, Caroline F. Frey, Nikola Pantchev, Walter Basso** Cutaneous abdominal biopsy enabled the diagnosis of clinical *Trichinella britovi* infection in a hunting dog
10:45-10:50	**P33. Gianluca Marucci, Alessia Possenti, Marilena Interisano, Simona Cherchi, Daniele Tonanzi, Alessandra Ludovisi, Federica Santolamazza, Azzurra Santoro, Paolo Vatta, Simone Cacciò, Maria Angeles Gómez-Morales** Activities of the European Union Reference Laboratory for Parasites (EURLP) on *Trichinella* during 2019-2022
10:50-10:55	**P34. Sasa Vasilev, Branko Suvajdzic, Nedjeljko Karabasil, Ljiljana Sabljic, Ivan Vicic , Ivana Mitic, Dragan Vasilev** Effectiveness of Priochek kit in laboratories performing *Trichinella* proficiency testing

Session I: Epidemiology and Human Trichinellosis

Trichinellosis in Serbia: Epidemiological trends and contributions of the National Reference Laboratory

Ivana Mitic[1*], Natasa Ilic[2], Sasa Vasilev[1], Alisa Gruden-Movsesijan[1], Sofija Glamoclija[1], Ljiljana Sabljic[1], Dragana Plavsa[3], Ljiljana Sofronic-Milosavljevic[1]

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Serbia, University of Belgrade, Belgrade, Serbia
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In Serbia, trichinellosis is a mandatory reportable disease, and the actual prevalence and incidence of *Trichinella* infection in humans is published every year by the Institute of Public Health of Serbia „Dr Milan Jovanovic Batut". Epidemiological data for the period of the last 10 years (2013-2022) show that sporadic cases or outbreaks occur almost every year (with exception of 2021) and indicate that for the last 5 years (2018-2022) the number of patients has decreased significantly (102 in comparison with 468 for the period of the previous 5 years, respectively). Out of the total number of 570 registered cases of trichinellosis, only one quarter of blood samples (27.5% i.e. 157) reached the NRLT-INEP for confirmation of positive serology. Since trichinellosis has been present in Serbia for decades, medical doctors, especially in endemic areas, have extensive experience and can suspect the disease in a timely manner. Serum samples are usually sent to local laboratories that use either imported Enzyme Linked Immunosorbent Assay (ELISA) or an indirect immunofluorescence assay (IFA) manufactured in Serbia (INEP, Belgrade). In cases where routine serodiagnosis did not allow a definitive diagnosis, sera were sent to NRLT-INEP.

Out of the 157 suspected cases referred to the NRLT INEP during ten-year period, positive anti-*Trichinella* antibodies were detected in 134 cases (85.3%), and Western blot (Wb) was performed as a confirmatory test in 14 cases due to discrepancies between the results obtained by IFA and ELISA. The NRLT significantly contributed to important observations at the national level by enabling: 1. Better insight into the response to *Trichinella britovi* infection in the large outbreak in 2016 (Pavic et al., 2020); 2. Assessment of the possible longevity of the presence of specific antibodies in human sera after infection with *Trichinella spiralis* (at least 18 years, Ilic et al., 2022) 3. Monitoring, not only the humoral, but also the specific cellular response in the trichinellosis outbreak that occurred during the COVID-19 pandemic in 2022, and 4. Evidence that the application of the One Health concept could significantly contribute to achieving better infection control and reducing the presence of disease in humans (Vasilev et al., 2023). At the international level, there is cooperation with reference laboratories in the EU regarding: 1. Trace back study of trichinellosis exported to France in 2017 (Barruet et al., 2020), 2. Regular participation at Workshops and PT schemes organized by EURLP, ISS, Rome, It. (Funded by Ministry of Science, Technological Development and Innovation, Republic of Serbia, Co. No. 451-03-47/2023-01/200019)

Keywords: trichinellosis, Reference Laboratory, Serbia

Session I: Epidemi

Human trichinello

Authors: Davor Balić
Ana Majić[2], Iva Pem-

Affiliations:

1- Croatian Veterinar
24, 32100 Vinkovci,

2- Croatian Institute
Croatia

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Session I: Epidemiology and Human Trichinellosis

Trichinella infection in Serbia, from 2019 to 2022

Sasa Vasilev[1*], Ivana Mitic [1], Dragana Plavsa [2], Ljiljana Sabljic[1], Milorad Mirilovic[3], Milos Petrovic[4], Budimir Plavsic[5], Ljiljana Sofronic-Milosavljevic[1]

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During the period from 2019 to 2022, approximately 2.3 million pigs were slaughtered annually in Serbia and examined in slaughterhouses, veterinary ambulances, stations and Institutes for the presence of *Trichinella* larvae in the meat. The average rate of domestic swine infection was 0.003%. This represents a steady improvement compared to the previous five-year periods (for which the average infection rate value were: 0.005% in the period 2014-2018 and 0.018% in 2009-2013). The prevalence of infection in wild boar was 0.911% in 2014-2018 and remained similar in the period 2019-2022. The number of *Trichinella* positive backyard pigs, recognized as the main source of trichinellosis in Serbia, has been decreasing over the years, as has the number of infected people. While for the period 2013-2017 there were 468 cases of human infection, a significant decrease was observed for the period 2018-2022 (102 cases). In all human cases, the source was *Trichinella* containing meat and/or meat products prepared without parasitological examination. Homemade meat products intended for personal use were often shared among relatives and friends in Serbia and abroad, representing a highly appreciated but dangerous gift if it is prepared from untested and infected meat.

The decrease in annual outbreaks of trichinellosis indicates an increased awareness of the risk of the disease and may be a consequence of the enforcement of the measures introduced in education and prevention. Hunters and backyard swine meat consumers in Serbia should be continuously educated about the risk associated with the consumption of untested meat. At the same time, both control of *Trichinella* testing QA system in veterinary subjects and regular participation in PTs are necessary to achieve safe food for consumers. (This work is supported by Ministry of Science, Technological Development and Innovation, Republic of Serbia, contract number: 451-03-9/2023-14/ 200019)

Keywords: *Trichinella spp. infection,* Serbia

Session IV: Biology, Host-Pathogen-Interaction and Immunology

Can we use Trichinella spiralis muscle larvae extracellular vesicles for the treatment of allergic airway inflammation?

Sofija Glamočlija[1*], Anna Schmid[2], Nataša Ilić[3], Alisa Gruden-Movsesijan[1], Ljiljana Sabljić[1], Saša Vasilev[1], Irma Schabussova[2], Maja Kosanović[1]

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Extracelular vesicles (EVs) are a fundamental way of communication between cells of one organism but also between different organisms and even different species. These nano-sized membrane-limited biological particles, carry information in the form of proteins, lipids, RNA, as well as other molecules, and deliver them to target cells, thus playing role in all physiological and pathophysiological processes in an organism. We have recently discovered that extracellular vesicles isolated *from Trichinella spiralis* excretory-secretory products (TsEVs) exert immunomodulatory properties on human monocyte derived dendritic cells. It was previously shown that *T. spiralis* infection alters immune response and exerts beneficial effect in mice with allergic airway inflammation. Our goal was to investigate the potential of TsEVs to ameliorate ovalbumin (OVA)-induced allergy in a murine model. Experimental allergic airway inflammation was induced in BALB/c mice by intraperitoneal injection of OVA in alum. On days 21-24 mice were challenged with intranasal application of OVA. Treatment by intranasal administration of TsEVs was performed on the days of sensitization and challenge. Blood samples were taken for serum IgE determination, while lungs and spleens were extracted for the isolation of immune cells. Phenotype of immune cells was determined by flow cytometry.

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TsEVs treatment lead to significant lowering of the proportion of CD103+ dendritic cells (DC) in lungs compared to allergic control, cells that play important role in priming Th2 response to inhaled allergens.
On the other hand, the proportion of CD11b+ Ly6C+ subset of cells, i.e. myeloid-derived suppressor cells, was increased, along with CD8+ and CD19+ T cells. Upon restimulation with OVA, lung immune cells of TsEVs-treated mice produced lower level of Th2 cytokine IL-5, while the production of Th1 cytokine IFN-γ was elevated. Lower IgE levels were found in TsEVs-treated mice compared to sham-treated controls. Our preliminary results show that TsEVs possess immunomodulatory properties that are reflected in the alleviation of allergic airway inflammation in mice. These results encourage further research into possible application of vesicles for therapeutic purposes.
(Funded by Ministry of Science, Republic of Serbia, Co. No. 451-03-47/2023-01/200019)

Keywords: *Trichinella spiralis*, extracellular vesicles, allergy

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Session V: Detection, Legislation and Control

Effectiveness of Priochek kit in laboratories performing *Trichinella* proficiency testing

Sasa Vasilev[1*], Branko Suvajdzic[2], Nedjeljko Karabasil[2], Ljiljana Sabljic[1], Ivan Vicic[2], Ivana Mitic[1], Dragan Vasilev[2]

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Trichinellosis is an important worldwide foodborne zoonosis. The gold standard *Trichinella* test for meat intended for human consumption is the magnetic stirrer artificial digestion method based on the use of pepsin and hydrochloric acid. To assess the performance of laboratories for their conduct of the method, the proficiency testing is used. A relatively new equivalent method - *Trichinella* priochek AAD kit, which is based on the artificial digestion by serin-endopeptidase enzyme, is also recently allowed in Republic of Serbia. The aim of this study was to test the usefulness of *Trichinella* Priochek AAD kit in *Trichinella* proficiency testing (PT) compared to the reference artificial digestion method according to EU directive 1735/2015 and ISO/IEC 17043:2010. Proficiency samples of pork diaphragm containing specific numbers of *Trichinella spiralis* larvae (naked and enkapsulated) were tested independently in two Serbian laboratories (Faculty for veterinary medicine, Belgrade; and in National reference laboratory for *Trichinella*). The results revealed that *Trichinella* Priochek AAD kit was simple and convenient to use, and showed good larval recovery that met the requirements of the EU Directive and criterion for successful PT participation. Control group included samples digested by using pepsin according to EU directive 1735/2015, and this method showed better larvae recovery then the *Trichinella* Priochek AAD kit.

But regardless, the larvae recovery obtained by the *Trichinella* Priochek AAD kit was satisfactory and confirmed it's suitability for using in PTs. (This work is supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, contract numbers: 451-03-68/2023-14/ 200019 and 451-03-47/2023-01/200143)

Keywords: *Trichinella*, proficiency testing, Trichinella Priochek AAD kit

Session V: Detectio

The historical devel
Serbia and the role

Vesna Djordjevic[1], Ljil
Mirilovic[3], Nedjeljko K
Sasa Vasilev[2*]

[1]Institute of Meat Hygi
[2]University of Belgrac
INEP, Belgrade, Serbia
[3]University of Belgrad

*Presenting author: Sa

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Session V: Detection, Legislation and Control

The historical development of meat examination on *Trichinella* in Serbia and the role of dr Milovan Djordjevic

Vesna Djordjevic[1], Ljiljana Sofronic Milosavljevic[2], Marko Savic[1], Milorad Mirilovic[3], Nedjeljko Karabasil[3], Ivana Mitic[2], Alisa Gruden Movsesijan[2], Sasa Vasilev[2*]

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The first meat examination by compression method for presence of *Trichinella* larvae have been recorded in Yugoslavia in 1932. In the early 1980s, the Yugoslavia had a large number of industrial slaughterhouses producing meat and meat products. The Federal Committee for Agriculture was engaged in improving the situation in this area in cooperation with the Yugoslav Institute of Meat Technology. The leader of the cooperation was Dr Mirko Nad. Magnetic stirrer method has been introduced since 1984, by Milovan Djordjevic, Institute of Meat Hygiene and Technology, Belgrade. A large trichinellosis outbreak in Sremska Mitrovica (in 1985) had a significan impact on national regulatory decision makers to establish the Serbian Commission on Trichinelosis, as a joint body of the Ministry of Agriculture and the Ministry of Health of the Republic of Serbia. The members were Dr Ratibor Maric (Ministry of Health), Dr Ljubomir Nedic (Institute for Public Health of the Republic of Serbia), Dr Kosta Cuperlovic (INEP), Dr Milovan Djordjevic (Yugoslav Institute of Meat Technology), Dr Zlatibor Petrovic (Veterinary Faculty, Serbian Academy of Sciences and Arts), and Srboljub Pavlovic (Ministry of Agriculture). Among other tasks, over the years, the Commission conducted systematic review of the laboratories performing *Trichinella* examination in 10 specialist veterinary institutes and slaughterhouses.

The Commission significantly contributed to the improvement of pig production practices in general, efficacy of veterinary services and the prosperity of the country's economy including the reduction in the infection rate among swine from 0.09% in 1980 to 0.009–0.02% for the period 1980–1990. The re-emergence of this infection coincided with period (1990–1999) when political and social changes, and civil wars took place. The increased prevalence of infection in swine (up to 0.17% by 1999) was accompanied by a significantly higher number of cases of trichinellosis (more than 500 on average per year). The rate of swine infection decreased to 0.11% in 2001. The Serbian Commission on Trichinelosis was abolished in 2002. (This work is supported by Ministry of Science, Technological Development and Innovation, Republic of Serbia, contract numbers: 451-03-68/2023-14/ 200019 and 451-03-47/2023-01/200143).

Keywords: *Trichinella*, magnetic stirrer method, dr Milovan Djordjevic

Session V: Detection,

**Proficiency tests for d
Regulation (EU) 2015/
and results**

Jana Sachsenröder[1], Anne

[1]German Federal Institute
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Corresponding author: Jar

Keywords: proficiency tes

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When organizing PT bo
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CERTIFICATE OF PARTICIPATION

Dr. Sasa VASILEV

attended and participated in the

XVIth International Conference on Trichinellosis

August 30th - September 1st, 2023
in Belgrade, Serbia

Dr. Ljiljana Sofronic-Milosavljevic
Chair Local Organizing Committee