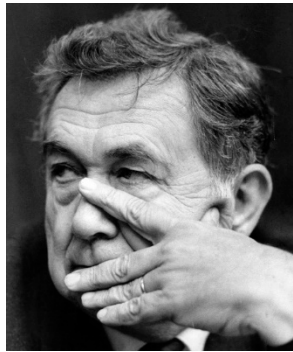


**The results of the NRL for Trichinellosis (NRLT-INEP):
Fifteen fruitful years of cooperation with the EURLP**

Sofronic-Milosavljevic Ljiljana

Reference Laboratory for Trichinellosis, NRLT-INEP
Department for immunology and immunoparasitology,
Institute for the Application of Nuclear Energy "INEP",
University of Belgrade, Belgrade, SERBIA

The origins of NRLT- Historical overview 1980 - 2007



**Dr Kosta
Cuperlovic**

1931-2005

Project leader,
INEP, Belgrade,
Serbia

Among Dr. Cuperlivic's collaborators



**Dr Milovan
Djordjevic**
1940-2018



**Dr L. Sofronic
Milosavljevic,**
participant, 1985-

1980-1990 The first systematic epizootiological investigations in Serbia (2 joint Scientific projects with USDA, USA:

1. Development of Immunodiagnostic Tests for Selected Helminth Parasites, 1981-1985
2. Detection, Epidemiology and Control of the Most Important Food Borne Parasite, 1986-1990

Murrell KD,



Gamble HR,



Marinculic A.



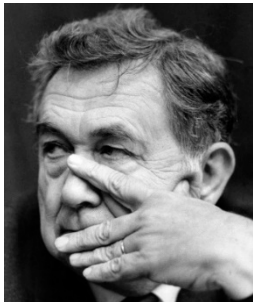
3. Magnetic stirrer method was introduced since 1984
4. Member of the Serbian Commission on Trichinelosis, established in 1985 (joint body of the Ministry of Agriculture and the Ministry of Health of the Republic of Serbia), contributed to the improvement of pig production practices in general including the reduction in the infection rate among swine from 0.09% in 1980 to 0.009–0.02%

Political upheavels in Serbia between 1991-1999



Colaboration with ISS, Rome, Edoardo Pozio – Trichinella species determination of Serbian isolates. Out of more than 100 isolates one, designated as ***T. spiralis* ISSN 161** was mainted at INEP until recently (publication Ilic et al. **2018**. *Front. Immunol.* 9:11, after which a **new isolate ISS 7564** was in use by the Serbian NRLT).

In 1993, by decision of the Ministry of Agriculture, FR Yugoslavia, INEP become the **Reference Laboratory for Trichinellosis and Brucellosis**, head **Dr K. Cuperlovic**. It was valid until **2006** when the country's name was changed to Republic of Serbia



Upon his retirement at 1997, Dr Cuperlovic K. continued to collaborate with L. Sofronic-Milosavljevic and colleagues at INEP, in Serbia and abroad until 2005.

Dr K. Cuperlovic published 35 joint scientific papapers with the scientists mentioned here

Historical review of the presence of *Trichinella* infection in animals and humans in Serbia

In Serbia, parasites of the *Trichinella* genus were detected initially in pig carcasses in 1918, while the first cases of human infection were recorded in 1923. The improvement in swine production practices had cut the prevalence of *Trichinella* infection in swine from 0.11% in 1918 to less than 0.009% in the 1980. (Cuperlovic et al., 1991, Southeast Asian J. Trop. Med. Public Health, 22:72) The re-emergence of trichinellosis in Serbia coincided with period (1990–1999) when political and social changes, and civil wars took place in the country. A period of next 10 years was needed to overcome that.

Veterinary Parasitology 194 (2013) 145–149

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journal homepage: www.elsevier.com/locate/vetpar



Trichinella infection in Serbia in the first decade of the twenty-first century

Lj. Sofronic-Milosavljevic^{a,*}, M. Djordjevic^{b,1}, B. Plavsic^c, B. Grgic^d

Epidemiology and Infection

cambridge.org/hyg

Trichinella infection in Serbia from 2011 to 2020: a success story in the field of One Health

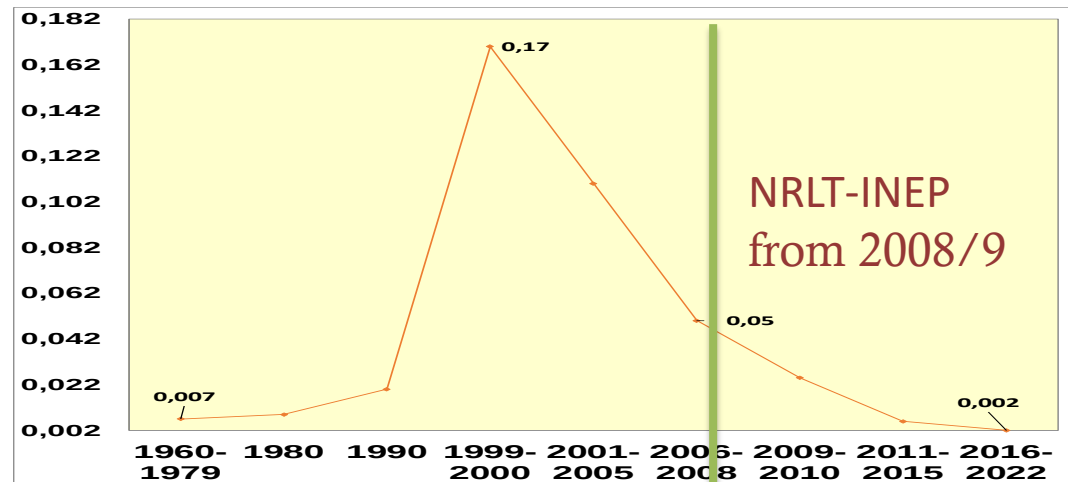
Sasa Vasilev^{a,*}, Ivana Mitic^{a,*}, Milorad Mirilovic^c, Dragana Plavska^a, Emina Milakara^d, Budimir Plavsic^d and Ljiljana Sofronic-Milosavljevic¹

^aNational Reference Laboratory for Trichinellosis, Institute for the Application of Nuclear Energy - INEP, University of Belgrade, Belgrade, Serbia; ^bFaculty of Veterinary Medicine, University of Belgrade, Belgrade, Serbia; ^cInstitute of Public Health of Serbia - Dr Milan Jovanovic Batut, Belgrade, Serbia; ^dMinistry of Agriculture, Forestry and Water Management of Serbia, Veterinary Directorate, Belgrade, Serbia and ^eWorld Organization for Animal Health (OIE), Paris, France

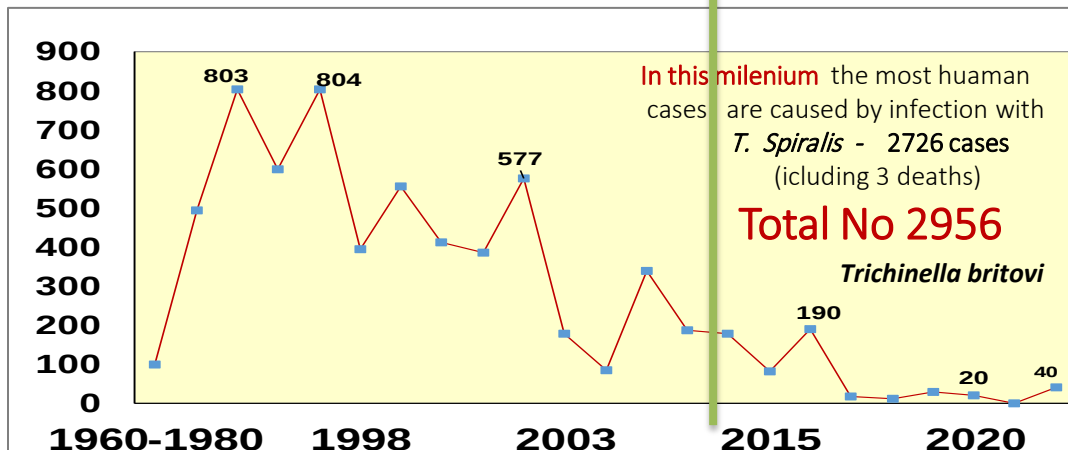
*These authors contributed equally to this work.

Cite this article: Vasilev S, Mitic I, Mirilovic M, Plavska D, Milakara E, Plavsic B, Sofronic-Milosavljevic L (2023). *Trichinella* infection in

Percentage and distribution of *Trichinella* infection in domestic pigs in Serbia (1960-2022)



Number of patients with trichinellosis in Serbia (1960 - 2022)



In 2007, INEP applied for the status of National Reference Laboratory to the Ministry of Health of the R. of Serbia. Call was part of a European project:



Strengthening the Services of Public Health Laboratories in Serbia
EU-funded project managed by the European Agency for Reconstruction



Under construction:

Referral system in Serbia

In the field of clinical microbiology carried out by laboratories there are up to now only few "unofficial" referral activities. A functioning Referral system will help Serbia fight against diseases such as tuberculosis, influenza, syphilis and salmonella. Particularly there are no referral activities in the field of hygiene.

Current weaknesses

- ❖ Insufficient definitive characterizations of infectious agents throughout the country (typing, resistance...)
- ❖ No organized system for disease confirmation and full characterization
- ❖ No availability of a national bacterial strain library
- ❖ Lack of surveys on national resistance patterns
- ❖ Lack of disease related data transmission for disease surveillance
- ❖ Lack of specialized disease-oriented supervision of laboratories
- ❖ Lack of international reputation of Public

Specific Objective:

Achieve reliable and comparable quality of laboratory services through the implementation of National referral system

What are Reference or Referral Laboratories?

- a. *In the field of Clinical microbiology* these are national public institutions which have been provided with a **time limited mission** related to one or several particular diseases
- b. *In the field of Hygiene* these are national public institutions related to samples or chemicals or even biological agents to monitor the quality of the environment, e.g. water

The mission is:

- ❖ Expertise in epidemiology, characterization of micro-organisms, sensitivity towards anti-infectious agents
- ❖ Epidemiological and hygienic surveillance
- ❖ Alert and information of responsible Ministries and other state authorities
- ❖ Participation in disease control and prevention programmes
- ❖ Communication and training

Proficiency testing (PT) issues

Inclusion in international PT networks; Help in PT policy development

Participation to International PT schemes; National PT unit creation

Meetings, symposiums and training activities

Participation to international meetings

Training on quality assurance, antibiotic susceptibility testing etc.

Use of QA-related software

Expected results

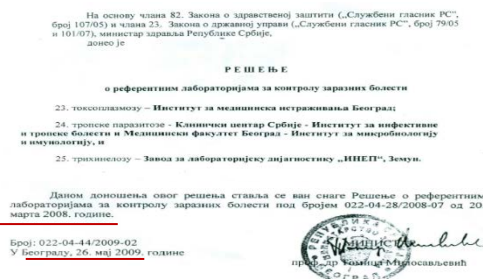
- Measurable, improved and adequate quality of analysis
- Alignment with EU and International standards
- Increased access to modern laboratory documentation
- Accreditation of laboratories
- Regular participation to PT schemes
- Increased international cooperation
- Increased cost-effectiveness



Strengthening the Services of Public Health Laboratories in Serbia
EU-funded project managed by the European Agency for Reconstruction



Add. Development of information services including websites, newsletters, open door days and more



April 22, 2008, amended May 2009

The Ministry of Health of R. Serbia declared
25 NRLs for Communicable Diseases, among
which INEP is recognized as the
National Reference Laboratory
for Trichinellosis (NRTL-INEP).



2008-2023 The Head of Serbian NRTL-INEP - Dr Ljiljana Sofronic-Milosavljevic

From August 2023 onwards The Head of NRTL-INEP – Dr Ivana Mitic

Sofronic-Milosavljevic Lj., 18th Workshop of the NRLs for Parasites, November 16-17, 2022, ISS, Rome, It

Topics of cooperation with EURLP

- ❖ Participation in scientific meetings (WS EURLP)
- ❖ Collection, processing and analysis of epizootiological and epidemiological data at national level accompanied by
- ❖ Annual reports and coordination existing at international level, *e.g.* involvement in EU and other international disease specific networks
- ❖ Participation in EU/international quality assessment schemes
- ❖ Participation in trainings through an EURLP open call
- ❖ Storage and distribution of standard strains, diagnostic standards, antigens
- ❖ Harmonization with new methods introduced in EURLP (molecular PCR tests, ELISA, microsatellites)
- ❖ Participation in collaborative studies

PARTICIPATION PARTICIPATION IN ERURLP WORKSHOPS

3rd Workshop of National Reference Laboratories for Parasite, Istituto Superiore di Santa, Rome, It., 29-30 May, 2008



The FIRST participation of Serbian NRLT at the invitation of the head of the Community Reference Laboratory for Parasites - Dr E. Pozio (CRLP later on become **European Union Reference Laboratory for Parasite – EURLP**, new head of EURLP - *Simone M Cacciò*, 2018 -).

The Serbian NRLT belongs to a small number of NRLTs outside the EU, and the cooperation in this field is still ongoing. It covered the picture of the presence of infection with *Trichinella* in human and animals (domestic swine and wild boars) in Serbia by regular reporting on WS in form of oral or poster presentations (From 2008-2023, with the exception of 2010, 2020 and 2021) as well as with the two publications mentioned before.

All of the workshop agendas from 2006 to 2018 are available as pdf documents. The presentations (slides or posters) are available upon request, while full content of the WS from 2019 -2022 is available at EURLP web site <https://www.iss.it/en/eurlp-workshops>

Sofronic-Milosavljevic Lj., 18th Workshop of the NRLs for Parasites, November 16-17, 2022, ISS, Rome, It



DELIVERY OF IMPROVED LOCAL SERVICES (DILS) PROJECT

The World Bank

Implementing Agencies:

Ministry of Health (MOH)

Ministry of Education, Science and Technological Development (MOESTD)

Ministry of Labor, Employment, Veterans and Social Policy (MOLEVSP)

National Reference Laboratory for Trichinellosis – INEP Laboratory

accredited by International standard ISO 17025:2006

Ljiljana Sofronić-Milosavljević

**Institute for the Application of Nuclear Energy "INEP", University of
Belgrade, Belgrade, Serbia**

Project "Improving the work of National Reference Laboratories"
Belgrade, 09.07.2013.

Annual reports and coordination existing at international level, e.g. involvement in EU and other international disease specific networks.

- **Annual reports** on Trichinellosis in Serbia has been **regularly provided by Serbian NRLT** to the:
- **1. European Union Reference Laboratory for Parasite – EURLP**, Istituto Superiore di Sanita, Rome, It., **where is also the seat of the International Trichinella Reference Laboratory - ITRL**, the official reference laboratory of both the International Commission on Trichinellosis –ICT (from 1998) and the World Organization for Animal Health – OIE (from 1992). Reports were in a form of oral or poster presentations
- **2. Annual reports in a written form to International Commission on Trichinellosis - ICT** (until 2013)
- The reports are based on passive surveillance since the most of data originated from the Department for control and prevention of communicable diseases, Institute of Public Health of Serbia “Milan Jovanovic Batut, only some of them by NRLT project collaboration with the Clinic for Infectious and Tropical Diseases, Clinical Center of Serbia, regional Institutes of Public Health and hospitals all over Serbia. The reports also include information on *Trichinella* infection prevalence among the swine and wild life (Collected either from the 25 districts’ veterinary authorities in Serbia by NRLT and with permission of the Serbian Ministry of Agriculture, Forestry and Water Management or in last couple years from its Veterinary Directorate)

Participation in EU/international quality assessment schemes

Serbian NRLT regularly participated in Proficiency test (PT) for the detection of *Trichinella* larvae in meat by Magnetic Stirrer Method which is organized annually by EURLP, ISSS

Serbian NRLT and Proficiency test for the detection of *Trichinella* larvae in meat by Magnetic Stirrer Method organized at national level



Veterinary Parasitology
Volume 320, August 2023, 109982



Trichinella proficiency testing in Southeastern European countries

[Sasa Vasilev](#)^a, [Laurentiu Mihai Ciupescu](#)^b, [Nikolay Lalkovski](#)^c, [Davor Balic](#)^d,
[Dragan Vasilev](#)^e, [Gianluca Marucci](#)^f

In Serbia PT was first organized in 2017.

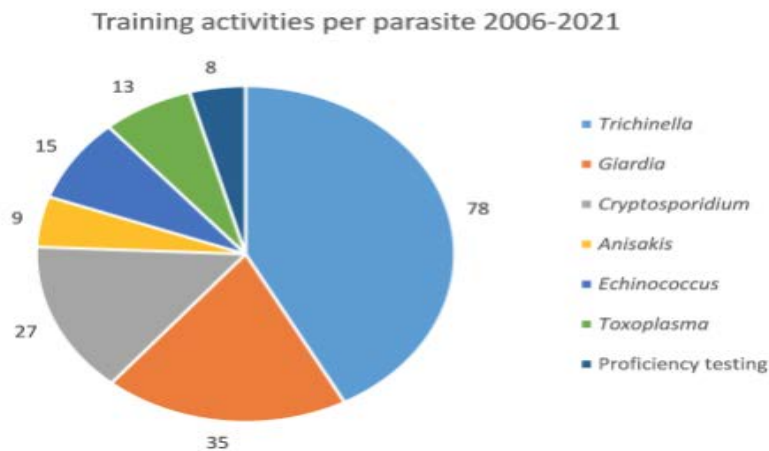
Publication presents data on the performance obtained by official laboratories of Southeastern European (SEE) countries that organize PT at national level. Results suggest that the constant participation in PT leads to an increase in the performance of participating laboratories by positively affecting the staff accuracy in sample testing by MSM.

[Gómez-Morales M.A.](#), [Ludovisi A.](#), [Pezzotti P.](#), [Amati M.](#), [Cherchi S.](#), [Lalle M.](#), [Pecoraro F.](#), [Pozio E.](#); [Ring Trial Participants](#) ([Glawischnig W.](#), [Dorny P.](#), [Gajadhar A.A.](#), [Economides C.](#), [Koudela B.](#), [Maddox-Hyttel C.](#), [Oksanen A.](#), [Vallée I.](#), [Noeckler K.](#), [Boutsini S.](#), [Sréter T.](#), [van der Giessen J.](#), [van Knapen F.](#), [Angelo H.](#), [Sofronic Milosavljevic L.](#), [Krt B.](#), [Perteguer M.J.](#), [Osterman-Lind E.](#), [Frey C.](#), [Hill D.E.](#)). (2009). **International ring trial to detect anti-*Trichinella* IgG by ELISA on pig sera.** *Veterinary Parasitology* 166: 241-248. doi: 10.1016/j.vetpar.2009.09.005

Sofronic-Milosavljevic Lj., 18th Workshop of the NRLs for Parasites, November 16-17, 2022, ISS, Rome, It

EURLP Training activities (2006-2021)

- These graphical representations illustrate the training activities organized during 2006-2021 by the specific pathogen and by participating countries.



Participation in trainings through an EURLP open call, Storage and distribution of standard strains, diagnostic standards, antigens; Harmonization with methods introduced in EURLP (molecular PCR tests, ELISA, microsatellites)

Education and visits of INEP scientists in the period 2009-2022

INEP scientists



Cvetkovic
Jelena,
27.09-10.2009.



Vasilev
Sasa,
07-10.09.2014



Gnjatovic
(Devic)
Marija, 2013



Sabljić
Ljiljana,
23-27.09.2019.



Glamoclija
Sofija,
03-07.10.2022

Topics for training activities or/and visits to EURLP

***Identification of *Trichinella* muscle larvae at species level by Multiplex PCR**

DOI: 10.2478/1168-4117-0025-1
© M. Sotirovic Institute of Parasitology, PAS
Acta Parasitologica, 2011, 56(2), 232-235, ISSN 1230-2821

VERSITA

RESEARCH NOTE

First report of *Trichinella britovi* in Serbia

Jelena Cvetkovic¹, Vlado Todorovic², Gianluca Marucci³, Dragan Vasilev⁴, Sasa Vasilev⁴,
Dusko Cirovic⁴ and Ljiljana Sofronic-Milosavljevic^{1*}

***Artificial digestion to detect *Trichinella* larvae in meat samples according to ISO 18743:2015, Organization of *Trichinella* PT**

Veterinarski Glasnik

Short Communications

<https://doi.org/10.2298/VETGL181220006V>

FIRST SERBIAN EXTERNAL QUALITY ASSESSMENT
TO DETECT *TRICHINELLA* LARVAE IN MEAT BY THE
MAGNETIC STIRRER METHOD

VASILEV Sasa^a, ILIC Natasa, SOFRONIC-MILOSAVLJEVIC Ljiljana

***Detection of anti-*Trichinella* IgG in serum from infected host by indirect ELISA, Collaboration on development of INEP ELISA tools and reference sera bank (2013-)**

Journal of Helminthology
© Cambridge University Press 2017
[cambridge.org/jhl](https://doi.org/10.1017/S0022267517000000)
Gnjatovic M et al.

A competitive enzyme-linked immunosorbent assay for rapid detection of antibodies against *Trichinella spiralis* and *T. britovi* – one test for humans and swine

***Animal facility conditions, Maintenance of *Trichinella* spp. in rodents, Cultivation muscle larvae for ES antigen production.**

***Identification of *Trichinella* spp. proteins recognized by specific IgG in serum of infected hosts by western blotting**

Mentored by



Gianluca
Marucci



Maria Angeles
Gomez Morales,
Alessandra Ludovisi



Simona Cherchi,
Maria Angeles
Gomez Morales,





Participation in collaborative studies

FP6-INCO-CT-043702- SERBPARTOON (2007-2009)

REINFORCEMENT OF THE SERBIAN CENTRE FOR PARASITIC ZOOSES



dr Sofronic
scientific visit to
EURLP,
FP6 project,
23.10.2009

- One of the specific objectives:
- Advanced training of SCPZ personnel provided by EU partners (new technology and methodology related)
- **EU NETWORKING PARTNERS**
 - **Edoardo POZIO, Ph.D.**
Director of the Unit for Gastroenteric and Tissue Parasite Diseases,
Department of Infectious, Parasitic and Immune-Mediated Diseases,
Istituto Superiore di Sanita, Roma, Italy
 - **Professor Jean DUPOUY-CAMET, M.D., Ph.D.** Unversite
Rene Descartes, Paris, France
 - **Maria ANTONIOU, B.Sc., ARCS, M.Sc., D.I.C., Ph.D**
University of Crete Faculty of Medicine, Heraklion, Greece
 - **Professor Richard GRENCIS, Ph.D**
University of Manchester Faculty of Life Sciences
Manchester, UK
 - **Philippe THULLIEZ, M.D.,** specialist in medical microbiology
Institut de Puericulture et Perinatologie de Paris
Paris, France

- NRLT INEP team expresses its gratitude to EURLP for all support provided during the last 15 years - since it has enabled NRLT INEP not only to be convinced of the competence of the work in the field of *Trichinella* and trichinellosis, but has also contributed to the acquirement of the new knowledge and practical experiences of researchers from INEP, Serbia.

Acknowledgement

- ❖ EURLP, ISS, Rome, Italy, all *Trichinella* experts
- ❖ Mitic I., Gruden-Movsesijan A., Glamoclija S., Sabljic Lj. **National Reference Laboratory for Trichinellosis NRLT-INEP**. Department for immunology and immunoparasitology, **Institute for the Application of Nuclear Energy - INEP**, University of Belgrade, Belgrade, Serbia
- ❖ Vasilev S. **National Reference Laboratory for the detection of *Trichinella* in the meat of domestic and wild animals** was appointed at Department for immunology and immunoparasitology, **Institute for the Application of Nuclear Energy - INEP**, University of Belgrade, Belgrade, Serbia by Veterinary Directory, Ministry of Agriculture, Forestry and Water Management of Serbia at **October 2022**,
- ❖ Zivojinovic M. **Veterinary Specialist Institute, Pozarevac**, Serbia
- ❖ Plavska D, Saponjic V. Department for Control and Prevention of Communicable Diseases, **Institute of Public Health of Serbia “Milan Jovanovic Batut”**, Belgrade, Serbia



International Conference on Trichinellosis

August 30 - September 1, 2023 Belgrade, Serbia

THANK YOU FOR YOUR ATTENTION!

