

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

Program and Abstract Book

16th International Conference on Trichinellosis

All authors are responsible for the content of their respective abstracts.

Institute for the Application of Nuclear Energy INEP,
University of Belgrade
Banatska 31b
11080 Belgrade
Serbia

Belgrade 2023
152 Pages
Number of copies 80
Unagraf Printing House

ISBN-978-86-918341-1-1
24/08/2023

*Published under authority and support of Ministry of Science, Technological
Development and Innovation, Republic of Serbia*

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Welcome Address



Sofronic-Milosavljevic Ljiljana
Chair, ICT-16 Local Organizing Committee



Marija Gnjatovic, PhD
INEP Director

In Serbia, infection with *Trichinella spp.* has been recognized as a human health and animal husbandry problem for almost a century.

From the 70s of the previous century until today, Institute for the Application of Nuclear Energy -INEP, University of Belgrade has been engaged in research on this topic, that is, the study of the parasite itself, the immune response it triggers and modulate, and development of different diagnostic tools for detecting infection in humans and animals. A number of doctoral and master's theses were completed and more than 100 scientific papers were published in domestic and international journals during this fifty-year period. Through the publications, we have shown that trichinellosis was a major health, social and economic problem in the times when political upheavals, wars and sanctions were present in Serbia during the nineties. In that period INEP was recognized for its excellence for detection of *Trichinella* larvae in meat and for *Trichinella* species determination in the first decade of this century. This year we are celebrating the fifteenth anniversary of the National Reference Laboratory for Trichinellosis (NRLT-INEP). In this millennium, the One Health concept was implemented in Serbia and currently, trichinellosis is successfully kept under control, as only individual cases occur annually. We sincerely believe that INEP has contributed with its work in this field.

We are proud to organize and host the 16th International Conference on Trichinellosis and to continue the good tradition from previous international conferences which were held all over the world - from 15th conference held in Cluj-Napoca (Romania) over Berlin (Germany), Changchun (China), Plitvice Lakes (Croatia), San Diego (USA) and Fontainebleau (France) in this millennium.

From August 30 – September 1, 2023 scientists from INEP will do their best to ensure your pleasant stay in Belgrade and a successful conference at Mona Plaza Hotel. It is a great honor and pleasure to welcome the *Trichinella* research community and we wish us all successful and inspiring meeting.

Welcome Address

Ass.prof.dr. Milos Petrovic

**Acting Director of the Veterinary
Directorate**

**Ministry of Agriculture, Forestry
and Water Management**

Serbia



Based on the data from the reports of the European zoonoses monitoring institutions such as EFSA and ECDC, in addition to the other foodborne diseases, trichinosis still occupies a significant place in human infection.

Frequent movement of people, animals and goods, facilitated trade between countries, as well as increasing demands for the consumption of traditional or raw food, especially the trend of using novel food, such as the meat of reptiles or other exotic animals, lead to new risks in the transmission of pathogens, where parasitosis gain significant importance.

In this light, I welcome you to the XVI International Conference on Trichinellosis, organized by the Institute for the Application of Nuclear Energy "INEP", University of Belgrade, which gathers the most eminent international and European experts in this field, that will have the opportunity to exchange and share knowledge in the field of diagnosis, epidemiology and prevention of the spread of *Trichinella*.

As the Republic of Serbia follows the latest legislation and research in this area, new knowledge will contribute to the improvement of the entire field of "One Health concept" in the country.

I wish to all participants of the conference successful work during the sessions, a high-quality exchange of experiences and knowledge, with the aim of improving cooperation and preventing the spread of this important zoonosis.

Welcome Address

Verica Jovanovic
MD, MSc, PhD

Associate Professor, Acting Director
Institute of Public Health of Serbia
"Dr Milan Jovanović Batut"



Despite the downward trends reported in many countries in Europe and beyond, Trichinellosis as a worldwide food-borne parasitic zoonosis still presents a challenge for public health worldwide. Nowadays the establishment and implementation of the One Health concept is critical for insuring an effective response to the zoonotic health threats and food safety. Trichinellosis exemplifies one of the main One Health issues where zoonoses are concerned. Zoonotic disease prevention and control is a two-way street and it calls for stable communication between the human and animal health sectors. Within the public health sector, countries ought to strengthen capacities for early disease detection, diagnosis and treatment, all the while raising awareness on the risks of transmission throughout the population, as efficient primordial and primary prevention. Simultaneously, the animal health sector ought to fortify strategies for infection control in order to help preclude animal-to-human transmission.

Therefore, special attention should be drawn to the development and implementation programs for Trichinellosis control and eradication.

I would like to take this opportunity to wish you all a warm welcome, both on behalf of the National Institute for Public Health “Milan Jovanovic Batut” and myself personally, to the 16th International Conference on Trichinellosis, organized by the Institute for the Application of Nuclear Energy "INEP" and the International Commission on Trichinellosis in Belgrade this year. There is no doubt in my mind that the following three days will be filled with illuminating presentations, stimulating discussions and useful conclusions regarding the overall impact of Trichinella infections and how to best combat them in a professional setting.

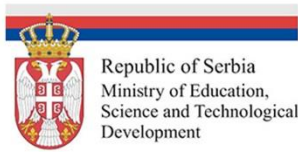
I wish you all prolific discussions, some take-away ideas to improve on back home and much luck in lessening the health burden that is Trichinella infection together!

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ACKNOWLEDGEMENT

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1 Program of the 16th International Conference on Trichinellosis

1.1 Program for oral presentations

Wednesday, 30th August 2023

-
- 12:00-12:30** **Plenary Lecture: Karsten Nöckler (Germany)**
From discovery of *Trichinella* to control of trichinellosis in the 21st century
-
- 14:00-15:00** **Session I: Epidemiology and Human Trichinellosis (Block I, Keynote lectures)**
Chairs: Joke van der Giessen (Netherlands), Mabel Ribicich (Argentina)
-
- 14:00-14:30** **Keynote: María Ángeles Gómez-Morales (Italy)**
Serological testing for epidemiological studies on *Trichinella* infection in animals and man
-
- 14:30-15:00** **Keynote: Fabrizio Bruschi (Italy)**
New immunological markers of human trichinellosis
-
- 15:00-16:00** **Session I: Epidemiology and Human Trichinellosis (Block II, Oral presentations)**
Chairs: Milos Korac (Serbia), Antti Oksanen (Finland)
-
- 15:00-15:15** **Ewa Bilska-Zajac, Benjamin Rosenthal, Peter Thompson**
How to trace inbred parasite outbreaks? Use Trich-tracker.
-
- 15:15-15:30** **Zuzana Hurníková, Yaroslav Syrota, Martina Miterpáková**
Contribution to the host diversity, genetic diversion, and epidemiology of *Trichinella pseudospiralis* in Slovakia, Central Europe
-
- 15:30-15:45** **Hélène Yera**
Human trichinellosis in France, 2000-2022
-
- 15:45-16:00** **Rajnish Sharma, Brad Scandrett, Edoardo Pozio, Jean Dupouy-Camet, Hélène Yera, Balbir Bagicha, Singh, Virak Khieu, Sotharith Bory, Emily Jenkins**
Trichinella spp in animals and humans of Southeast and South Asia: Status and gaps

Thursday, 31st August 2023

- 08:00-09:15** **Session II: Multidisciplinary, China**
Chairs: Ljiljana Sofronic-Milosavljevic (Serbia),
Isabelle Vallee (France)
-
- 08:00-08:15** **He Yushu, Xin Gao, Yong Yang, Xiaolei Liu, Fengyan Xu, Yang Wang, Lei Liu, Yaming Yang, Mingyuan Liu, Xue Bai**
Extracellular vesicles from *Trichinella spiralis*: Proteomic analysis and protective immunity. (Topic Immunology)
-
- 08:15-08:30** **Chengyao Li, Yi Liu, Xiaolei Liu, Xue Bai, Xuemin Jin, Fengyan Xu, Hong Chen, Yuanyuan Zhang, Isabelle Vallee, Mingyuan Liu, Yong Yang**
The gut microbiota contributes to changes in the host immune response induced by *Trichinella spiralis*. (Topic Immunology)
-
- 08:30-08:45** **Ruohang Sun, Xuemin Jin, Yi Liu, Jiaqi Wang, Xuelin Wang, Bin Tang, Mingyuan Liu, Xiaolei Liu**
 β -Glucan-triggered *Akkermansia muciniphila* expansion facilitates the expulsion of intestinal helminth via TLR2 in mice. (Topic Immunology)
-
- 08:45-09:00** **Wenjie Shi, Mingyuan Liu, Zhili Hao, Yue Liang, Xiaolei Liu, Ning Xu**
The immunosuppressive ability of *Trichinella spiralis* adults can ameliorate type 2 inflammation in murine allergy model. (Topic Immunology)
-
- 09:15-09:45** **Student Research Award**
Chairs: Brad Scandrett (Canada), Mabel Ribicich (Argentina)
- Jianda Pang, Xuemin Jin, Yi Liu, Zijian Dong, Jing Ding, Mingyuan Liu, Ning Xu, Xiaolei Liu**
Trichinella spiralis E3 ubiquitin ligase Ts-RNF ubiquitylates SQSTM1/p62 and inhibits myoblast differentiation to participate in cyst formation (Topic Host-pathogen interaction)

Thursday, 31st August 2023

10:15-11:20 Session III: Phylogeny, Genomics and Proteomics

Chairs: Ewa Bilaska-Zajac (Poland), Frits Franssen (Netherlands)

10:15-10:45 Keynote: Peter C. Thompson (USA)

Low-pass whole genome sequencing: a rapid means to trace the evolutionary history of *Trichinella spiralis*

10:45-11:05 Asis Khan, Matthew Valente, Subodh K. Srivastava, Vasin M. Fournet, Peter C. Thompson, M. Rosenthal

Long read sequencing vastly improves assembly of *Trichinella* genomes: updates and predictions.

11:30-12:30 Session IV: Biology, Host-Pathogen-Interaction and Immunology (Block I Keynote)

Chairs: Isabelle Vallee (France), Fabricio Bruschi (Italy)

11:30-12:00 Keynote: Zhiliang Wu (Japan)

Roles of *Trichinella*-secreted extracellular vesicles in the immune evasion and parasitism

12:00-12:30 Keynote: Alisa Gruden-Movsesijan (Serbia)

Immunomodulatory potential of *Trichinella spiralis* antigens: possible solutions for inflammatory disorders

14:00-14:40 Session IV: Biology, Host-Pathogen-Interaction and Immunology (Block II, Oral presentations)

Chairs: Maria Angeles Gómez-Morales (Italy), Alisa Gruden Movsesijan (Serbia)

14:00-14:20 Sofija Glamočlija, Natasa Ilić, Alisa Gruden-Movsesijan, Ljiljana Sabljic, Sergej Tomić, Ljiljana Sofronić-Milosavljević, Maja Kosanović

Do extracellular vesicles from *Trichinella spiralis* muscle larvae harbor the potential to induce regulatory T cells?

Thursday, 31st August 2023

- 14:20-14:40** **Ljiljana Sofronic-Milosavljevic, Alisa Gruden-Movsesijan, Natasa Ilic, Sergej Tomic, Sofija Glamoclija, Ljiljana Sabljic, Dragana Plavska, Nadezda Stankovic, Ljiljana Stevanovic, Nedeljko Vasic, Milena Zivojinovic, Ivana Mitic**
Influence of the specific immune response to SARS-CoV-2 virus antigens on the immune response to *Trichinella spiralis* antigens and vice versa

Friday, 1st September 2023

09:00-10:15 **Session V: Detection, Legislation and Control (Block I)**
Chairs: Karsten Nöckler (Germany), Brad Scandrett (Canada)

09:00-09:30 **Keynote: Joke van der Giessen (Netherlands)**
Importance of food-borne parasites in a One Health setting

09:30-09:45 **Sebastian Míguez, Adrián Moreno María, Fernando A Fariña, Mariana I Pasqualetti, Mabel M Ribicich**
Understanding the Global Connection: investigating the association between pork meat exports and trichinellosis

09:45-10:00 **Annika Boulaaba, Helga Nagengast**
Laboratories for *Trichinella* testing: Concept of surveillance in Lower Saxony and insights from the audits

10:00-10:15 **Isabelle Vallée, Aurélie Chevillot, Amandine Blaizot, Aurélie Heckmann, Laura Lucia Estevez, Michel Laurentie, Maud Marsot**
Analysis of historical data for *Trichinella* proficiency testing: towards the definition of performance evaluation standards for routine laboratories

10:15-10:30 **Fernando A Fariña, Mariana I Pasqualetti, Silvio J Krivokapich, Graciana Gatti, Tatiana Aronowicz, Franco J Laurito, Mariano E Ercole, Clara Bessi, Francisco Montalvo, Mabel M Ribicich**
Trichinella patagoniensis isolation from naturally infected puma from Neuquén province, Argentina

Friday, 1st September 2023

11:25-12:00 **Session V: Detection, Legislation and Control (Block III)**
Chairs: Brad Scandrett (Canada), Annika Boulaaba
(Germany)

11:25-11:40 **Walter Basso, Gastón Moré, Diana Gliga, Marie-Pierre
Ryser-Degiorgis, Norbert Müller, Caroline F. Frey**
Trichinella britovi and *T. spiralis* infections in large
carnivores and other wild species in Switzerland

11:40-11:55 **Frits Franssen F**
Quantitative microbial risk analysis is a useful
mathematical tool to evaluate relative risks regarding
foodborne parasites

11:55-12:00 **Benjamin Rosenthal and Valsin Fournet**
Pork Quality Assurance in the United States: evidence of
negligible risk

12:00-12:30 **Plenary Lecture: Dante Zarlenga (USA)**
Trichinella; becoming a parasite

1.2 Program for poster presentations

Wednesday, 30th August 2023

-
- 16:30-17:30** **Session I: Epidemiology and Human Trichinellosis (Block III, Flash poster presentations)**
Chairs: Zuzana Hurnikova (Slovakia), Milena Zivojinovic (Serbia)
-
- 16:30-16:35** **P1. Marina Winter, Sergio D Abate, Naira Klopertanz, Mabel M Ribicich:**
Surveillance in terrestrial and marine wildlife of Patagonia.
-
- 16:35-16:40** **P2. Zuzana Hurníková, Ali Halajian, Martina Miterpáková, Kgethedi Michael Rampedi, Yaroslav Syrota**
Trichinella (Nematoda) in South Africa, the possibility of a mesocarnivore (Mongoose) playing a role in the sylvatic cycle.
-
- 16:40-16:45** **P3. Annette Johne, Jana Sachsenröder, Karsten Nöckler, Nora Thaben, Martin Richter, Anne Mayer-Scholl**
Trichinella spp. in Germany – current situation in wildlife
-
- 16:45-16:50** **P4. Bretislav Koudela, Jiri Harna, Martin Pijáček**
Trichinella spp. findings in European badgers in the Czech Republic.
-
- 16:50-16:55** **P5. Janez Posedi, Maja Gorisek**
First detection of *Trichinella nativa* in foxes (*Vulpes vulpes*) in Slovenia.
-
- 16:55-17:00** **P6. Davor Balić, Gianluca Marucci, Marija Krajina, Miroslav Benić, Željko Mihaljević, Tibor Andreanszky, Tomislav Sukalić, Damir Lukačević, Tihana Miškić, Marica Lolić, Mario Škrivanko:**
Trichinella infection in two populations of golden jackal (*Canis aureus*) in Croatia.

Wednesday, 30th August 2023

- 17:00-17:05** **P7. Nino Iashvili, Nora Kokaia, Lela Arabuli:**
About the spread of trichinellosis in Georgia in the 21st century.
-
- 17:05-17:10** **P8. Maria Angeles Gómez-Morales, Alessandra Ludovisi, Marilena Interisano, Simona Cherchi, Daniele Tonanzi, Gianluca Marucci:** *Trichinella* infections in animals and humans in Italy from 2019 to 2022.
-
- 17:10-17:15** **P9. Călin Mircea Gherman, Zsolt Boros, Mihai-Horia Băieș, Anamaria Cozma-Petruț, Vasile Cozma**
Trichinella species infection in Romanian wildlife, a review
-
- 17:15-17:20** **P10. Ana-Maria Marin, Gheorghe Dărăbuș, D.C. Popovici, Gianluca Marucci, Calin Mircea Gherman, Narcisa Mederle**
Trichinella britovi – still a permanent presence in wild carnivores from the Banat area of Romania
-
- 17:20-18:05** **Session I: Epidemiology and Human Trichinellosis (Block III, Flash poster presentations):**
Chairs: Dragana Plavska (Serbia), H  l  ne Yera (France)
-
- 17:20-17:25** **P11. Elisa Di Maggio, Martina Meola, Vittorio Bramante, Maria Nesta, Michele Corritore, Francesca Fortunato, Rosa Prato, Domenico Martinelli, Emmanouil Alexandros Fotakis, Martina Del Manso, Patrizio Pezzotti, Christina Merakou, Alessandra Ludovisi, Mar  a Angeles G  mez Morales:**
Investigation of a trichinellosis Outbreak in Apulia region, Italy, February-March 2023

Wednesday, 30th August 2023

17:25-17:30 P12. Maura Fiamma, Alessandra Ludovisi, Ennio Bandino, Scilla Mastrandrea, Maria Cristina Garau, Anna Calvisi, Angelo D. Palmas, Maria Angeles Gómez-Morales
A trichinellosis outbreak in Sardinia that could have gone unnoticed

17:30-17:35 P13. Ivana Mitic, Natasa Ilic, Sasa Vasilev, Alisa Gruden-Movsesijan, Sofija Glamoclija, Ljiljana Sabljic, Dragana Plavska, Ljiljana Sofronic-Milosavljevic
Trichinellosis in Serbia: Epidemiological trends and contributions of the National Reference Laboratory

17:35-17:40 P14. Davor Balić, Sanja Kurečić Filipović, Ivan Mlinarić, Maja Ilić, Ana Majić, Iva Pem-Novosel
Human trichinellosis in Croatia: a review of the last 17 years

17:40-17:45 P15. D Vuchev, G. Popova
Dynamics of trichinellosis in humans in Central South Bulgaria (1990-2023)

17:45-17:50 P16. D. Vuchev, G. Popova, K. Anichin
On the treatment and chemoprophylaxis of trichinellosis

17:50-17:55 P17. D. Vuchev, G. Popova, A. Ivanova
An outbreak of trichinosis caused by *Trichinella britovi*

17:55-18:00 P18. Dragana Plavska, Vladan Saponjic, Verica Jovanovic, Marija Milic, Dragana Dimitrijevic, Milunka Milinkovic, Jovanka Cosic
Human trichinellosis in Serbia from 2001 to 2022

18:00-18:05 P19. Sasa Vasilev, Ivana Mitic, Dragana Plavska, Ljiljana Sabljic, Milorad Mirilovic, Milos Petrovic, Budimir Plavsic, Ljiljana Sofronic-Milosavljevic
Trichinella infection in Serbia, from 2019 to 2022

Thursday, 31st August 2023

08:00-09:15 Session II: Multidisciplinary, China

Chairs: Ljiljana Sofronic-Milosavljevic (Serbia), Isabelle Vallee (France)

09:00-09:05 P.20. Huifang Bai, Xiaolei Liu, Jing Ding, Bin Tang, Saining Wang, Shuyan Zhang, Ning Jiang, Xiaoxia Wu, Guoliang Chen, Qianqian Dang, Mingyuan Liu, Xuelin Wang

Isolation and identification of BALB/c mice skeletal muscle satellite cells and preliminary study on regulation in immune microenvironment in *Trichinella spiralis* infection. (Topic Biology, **Flash poster**)

09:05-09:10 P.21. Yao Yu, Xiaolei Liu, Lianjing Zhao, Xiuqin Chen, Ning Xu, Lin Li, Mingyuan Liu, Xue Bai

Colorimetric immunoassay via smartphone for *Trichinella spiralis* in human sera. (Topic Detection, **Flash poster**)

10:15-11:20 Session III: Phylogeny, Genomics and Proteomics

Chairs: Ewa Bilaska-Zajac (Poland), Frits Franssen (Netherlands)

11:05-11:10 P.22. Vladislav A. Lobanov, Kelly A. Konecni, W. Brad Scandrett, Emily J. Jenkins

Identification of *Trichinella* taxa by ITS-1 amplicon next-generation sequencing with higher sensitivity for under-represented species/genotypes in mixed infections

11:10-11:15 P.23. Azzurra Santoro, Simona Cherchi, Giuseppe La Rosa

Gene recombination and infectious dose defines the genetic structure of *Trichinella* isolates: experimental evidences. (**Flash poster presentation**)

Thursday, 31st August 2023

14:40-15:15 Session IV: Biology, Host-Pathogen-Interaction and Immunology

(Block III, Flash Poster presentations)

Chairs: Fernando A. Farina (Argentina), Caroline Frey (Switzerland)

14:40-14:45 P24. Clara Bessi, Mariano E. Ercole, Fernando A. Fariña, Francisco Montalvo, Valeria Fassa, Marcelo Acerbo, Mabel M. Ribicich, Mariana I. Pasqualetti
Survival of *T. spiralis* and *T. pseudospiralis* in experimentally infected wild boar muscle tissue under freezing and environmental conditions.

14:45-14:50 P25. Francisco Montalvo, Camila Mendoza, Clara Bessi, Fernando A. Fariña, Mabel M. Ribicich, Alejandra Volpedo, Mariana I. Pasqualetti
Comparison of the adult stage distribution pattern of three *Trichinella* species during the intestinal phase in CF1 mice.

14:50-14:55 P26. Orken S. Akibekov, Fariza S. Zhagipar, Alfiya S. Syzdykova, Aibek Kh. Zhumalin, Aissarat M. Gajimuradova
Comparative analysis of early diagnosis of invasion by species *Trichinella nativa* and *Trichinella spiralis* by serological and biochemical parameters

14:55-15:00 P27. Sofija Glamočlija, Anna Schmid, Nataša Ilić, Alisa Gruden-Movsesijan, Ljiljana Sabljčić, Saša Vasilev, Irma Schabussova, Maja Kosanović
Can we use *Trichinella spiralis* muscle larvae extracellular vesicles for the treatment of allergic airway inflammation?

15:00-15:05 P28. Marie Maurer, Elaa Chkhaydem, Amandine Blaizot, Laura-Lucia Estevez, Damien Destouches, Isabelle Vallee
Enrichment and characterization of muscle extracellular vesicles during *Trichinella spiralis* infection in mice

Thursday, 31st August 2023

In paper form
only, no oral
presentation

**P29. Zhiliang Wu, Sukhonthip Khueangchiangkhwang,
Yoichi Maekawa**

Trichinella spiralis muscle larva-derived deoxyribonuclease
II promoted differentiation of C2C12 myoblasts

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, Konecni 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 Vivic, Ivana Mitic, Dragan Vasilev**
Effectiveness of Priocheck kit in laboratories performing *Trichinella* proficiency testing

Friday, 1st September 2023

10:55-11:00 P35. Vesna Djordjevic, Ljiljana Sofronic-Milosavljevic, Marko Savic, Milorad Mirilovic, Nedjeljko Karabasil, Ivana Mitic, Alisa Gruden Movsesijan, Sasa Vasilev
The historical development of meat examination on *Trichinella* in Serbia and the role of dr Milovan Djordjevic

11:00-11:05 P36. Jana Sachsenröder, Anne Mayer-Scholl, Annette Johne
Proficiency tests for detection of *Trichinella spiralis* according to Regulation (EU) 2015/1375 in Germany – challenges, organization and results

In paper form only, no oral presentation

P37. Zhiliang Wu, Sukhonthip Khueangchiangkhwang, Yoichi Maekawa
Approach of early diagnosis of *Trichinella* infection by detecting microRNAs of extracellular vesicles

2 Abstracts for plenary, keynote and oral presentations

Plenary

From discovery of *Trichinella* to control of trichinellosis in the 21st century

Karsten Nöckler

German Federal Institute for Risk Assessment, Biological Safety, Berlin, Germany

Nematodes of the genus *Trichinella* can cause human trichinellosis, a disease characterized by gastrointestinal and muscular symptoms. After discovery of *Trichinella* in a patient by the English medical student James Paget and the pathologist Richard Owen in 1835, the complete life cycle of *Trichinella* was discovered by the German scientists Friedrich Zenker and Rudolf Virchow in 1860. They have demonstrated the link between *Trichinella* infection in pigs and human trichinellosis after consumption of raw or undercooked pork. Therefore as early as 1864, Virchow initiated the implementation of *Trichinella* meat inspection of pigs in the first German slaughterhouses by the microscopic examination of small muscle pieces namely trichinoscopy. Later, food safety authorities around the world began to introduce meat inspection programs in relevant domestic as well as wild animals to identify and prevent the spread of trichinellosis.

After the first description of the species *Trichinella spiralis* (Railliet, 1895), numerous steps were achieved in the understanding of the epidemiology of *Trichinella* and its adaption to different geographic conditions. Nowadays, 10 *Trichinella* species with 13 *Trichinella* genotypes have been described infesting a broad host spectrum of mammals but also birds and reptiles what live in the domestic and/or sylvatic cycle.

In line with taxonomic investigations and optimization of meat inspection to improve consumer protection, methods for detection and characterization of *Trichinella* larvae have been further optimized, developed, validated and standardised. In the 1970s, the trichinoscopy was replaced by the magnetic stirrer method what enables a much higher amount of muscle tissue to be tested for the pooled sampling approach during meat inspection.

As for other food-borne pathogens, improvements of direct detection methods have been accompanied by molecular methods such as PCR and mass spectroscopy in term of a fast and specific characterisation of isolated *Trichinella* larvae for epidemiological studies and outbreak investigations.

Today, trichinellosis is relatively rare in many countries due to implemented meat inspection systems and improved processing of meat and products thereof to inactivate *Trichinella* larvae such as cooking, irradiation and freezing (for meat from domestic pigs). The International Commission on Trichinellosis has published recommendations on the post-harvest control of *Trichinella* in food animals. In some countries, efforts have been made for *Trichinella*-free pork production systems to reduce the risk of *Trichinella* in pork by implementing measures such as improved animal husbandry.

Keywords: *Trichinella*, trichinellosis, history, control

2.1 Session I: Epidemiology and Human Trichinellosis

Keynote

Serological testing for epidemiological studies on *Trichinella* infections in animals and man

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Serological testing is widely used for detection of *Trichinella* infections in animals and humans, however it is not always possible to discriminate between exposed and non-exposed hosts with complete confidence. In humans, the detection of specific antibody (IgG) to confirm a suspected clinical diagnosis of trichinellosis is a valuable diagnostic tool and is considered to provide a similar or even better diagnosis in terms of sensitivity than the muscle biopsy. Conversely, considering the lack of predictability of antibody responses in animals, serological assays should not be used to detect *Trichinella* sp. infection in individual animals (carcass) for protecting human health. Nevertheless, antibody detection is suitable and appropriate for monitoring *Trichinella* infections in domestic animals and wildlife and can contribute to a better understanding of the epidemiology of *Trichinella* infection, especially when more than one test is used. The International Commission on Trichinellosis recommends using ELISA with excretory/secretory (ES) antigens, since these antigens are recognized by sera from hosts infected by all *Trichinella* species and genotypes identified thus far. Positive results obtained by ELISA should be confirmed by western blot. To increase the sensitivity of serological assays, significant progress has been made on the identification and characterization of antigens from pre-adult, adult and newborn *Trichinella* stages with the intent of finding molecules useful for serological testing of *Trichinella* infections during the early stages, when the use of ES antigens from muscle larvae generate false negative results. Several promising candidates for early detection have been listed and are waiting for validation in further studies. In terms of predictive values, the test performance is influenced by the prevalence of antibodies in the population.

The prevalence of detectable antibodies, in turn, depends on the rate of infection -with the age of the host and the time post infection- and antibody persistence, which, for *Trichinella* infections, depend on the species involved. Each serological test should be properly standardized and validated for its intended purpose.

Keywords: *Trichinella*, trichinellosis, serological testing, epidemiology, surveillance

Session I: Epidemiology and Human Trichinellosis

Keynote

New immunological markers for human trichinellosis

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Keywords: human trichinellosis, matrix metallo-protease 9, chemokines,
clinical signs < 5

Introduction

The immune response to *Trichinella* spp. was mainly studied in experimental infection in rodents where an early T helper (Th) 1 activation is followed by a stable Th2 polarization, whereas that, in human infections, particularly at cellular level, is poorly investigated. Few studies highlight a Th2 polarization or a mixed Th1-Th2 response. Furthermore, the mechanisms of the inflammatory response at either intestinal or skeletal muscle level are not known in human infection.

Chemokines (C-X-C or C-C) and matrix metalloproteinases (MMPs) are implicated in a number of parasitic infections such as malaria, neurocysticercosis, angiostrongyloidosis, and schistosomiasis, but poor is known about their role in human trichinellosis.

Aim of the study

For these reasons, we planned to study matrix MMP -2 and -9 as well as chemokines (CCL2 and CXCL10) in the sera of trichinellosis patients, with the aim to identify possible serological markers of the immune response activation and of the clinical involvement.

The above chemokines are in fact the expression of Th polarization, being CXCL10 the prototype of IFN- γ -inducible Th1 chemokines and CCL2 that of Th2 cell activation dependent chemokines.

Studied patients acquired infection by consuming raw sausages prepared with a mixture of wild boar and pork meat which resulted contaminated with *Trichinella britovi* larvae.

Serum MMP-9, but not MMP-2 levels resulted significantly increased in patients suffering relevant symptoms such as diarrhea, myalgia, and facial oedema. This result accounts for a potential reliable marker of inflammation of this enzyme in trichinellosis patients.

As regards chemokine levels, both CXCL10 and CCL2 levels resulted increased in sera collected during the acute phase of infection, then declining significantly in the convalescent phase.

A positive significant association was observed between MMP-9 and CXCL10 levels.

Furthermore, the CXCL10 level, but not that of CCL2, significantly correlated with the severity of symptoms being particularly higher in patients suffering diarrhea, myalgia, or facial oedema, as well as increased LDH and CPK levels.

Our results are in line with the previously described mixed polarization of Th cells in human trichinellosis.

In the light of these findings we suggest that CXCL10 and CCL2 might be considered as serological markers of Th polarization.

Session I: Epidemiology and Human Trichinellosis

How to trace inbred parasite outbreaks?

Use Trich-tracker.

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When a *Trichinella* infection is found in pigs, it is particularly important to identify the source of the infection for the animals and stop further transmission of the parasite to next pigs and into surrounding areas. For this, an epidemiological investigation is used, which is a complex procedure including few stages: epidemiological interview, serological investigations, species identification of discovered *Trichinella* larvae, and differentiation of isolates of the same species. The last step is practically not applicable due to the lack of available methods to distinguish isolates of *T. spiralis* larvae. And thus, in most cases, it was not possible to obtain an answer as to what could have caused the infection of pigs in a given outbreak of trichinellosis.

Here, we propose a method based on ddRADseq and bioinformatics analysis called - Trich-tracker. The methodology applies the ddRADseq technique during which DNA libraries are created using restriction enzymes. Such DNA libraries contain the sequences of multiple random loci distributed throughout the genome under analysis. The obtained in NGS raw sequence data is used for finding single nucleotide polymorphisms (SNPs) and then for phylogenetic analysis or genetic structure analysis. Both of the analyses are useful to distinguish isolates one from another, what is the most important during epidemiological investigation in *Trichinella* outbreaks on pigs' farms. The discriminating power of this tool is tunable and scalable, allowing application in a variety of epidemiological contexts.

The simplicity of the entire procedure, and the timeliness and cost effectiveness of Trich-tracker ensure the usefulness of its practical application in ongoing *Trichinella* outbreaks. Furthermore, Trich - tracker may be also used to track origin of cured meat containing *T. spiralis* which was a source of human infection. Additionally, this tool may be adapted to distinguishing other species of *Trichinella* and of any other parasites.

Session I: Epidemiology and Human Trichinellosis

Contribution to the host diversity, genetic diversion, and epidemiology of *Trichinella pseudospiralis* in Slovakia, Central Europe

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Trichinella pseudospiralis belongs to the non-encapsulated clade of the genus *Trichinella* and its epidemiology is influenced by various biotic and abiotic factors. Most *Trichinella*-infections occur in wildlife and the intensive urbanisation increases animal and human exposure to the parasite. Moreover, during the last decades, several non-native animal species were introduced to Europe, broadening the spectrum of the parasite reservoir hosts. Up to now, *T. pseudospiralis* was identified in 18 mammalian and eight avian species. The role of different animal species, and in particular birds, in the spread and epidemiology of the parasite is still not well understood and further research is needed in the areas where its occurrence has been recorded. For this purpose, molecular approaches and DNA sequencing represent valuable support.

In Slovakia, *T. pseudospiralis* was first recorded in 2004 on a poorly maintained industrial pig farm where pigs, rats, and a domestic cat were found to be infected. In the following years, its occurrence was confirmed in foxes, wild boars, and three species of birds of prey. In the present study the new host species, in particular, raccoon dog (*Nyctereutes procyonides*) and yellow-necked mouse (*Apodemus flavicollis*) together with results of the genetic structure study are presented and genetic structure conducted on a fragment of the 28S gene in *T. pseudospiralis* is analysed. The genetic study based on a haplotype network and Bayesian phylogeny analysis included 16 sequences of *T. pseudospiralis* available in the GenBank. The results indicate that geographic distance is a significant factor driving the observed genetic structure in contrast to species identity which was evaluated as not significant.

As *T. pseudospiralis* is the only species infecting both mammals and birds in Europe, and its spread is strongly affected by multiple factors including climatic changes and human activities, further epidemiological and genetic investigations are in demand.

Keywords: *Trichinella pseudospiralis*; Genetic analyses; Epidemiology; Parasitозoonoses

Acknowledgement: The study was funded by the Slovak Research and Development Agency under contract No. APVV-21-0166 and by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under project No. 09I03-03-V01-00046.

Session I: Epidemiology and Human Trichinellosis

Human trichinellosis in France, 2000-2022

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Trichinellosis is a foodborne parasitic disease transmitted after consumption of infected raw meat. In France, there were major outbreaks linked to horsemeat in the eighteenth-nineteenth. Then, the number of outbreaks decreased since the European obligation to control meat before human consumption.

So, from 2000 to 2022: there were small outbreaks mostly linked to the consumption of wild boar and imported meat. One hundred cases were reported, leading to a low incidence of trichinellosis. When regarding the origin of the cases, thirty percent acquired the infection abroad and forty-five percent consumed imported meat.

Trichinellosis is rare in France, but outbreaks within hunter-families are reminiscent of the circulation of *Trichinella* spp. in wild animals. Physicians should be informed of the risks of trichinellosis in hunter-families and in travelers. In addition, expertise in trichinellosis should be maintained to help the medical staff with the diagnosis of this disease.

Keywords: trichinellosis, *Trichinella*, human, France, epidemiology

Session I: Epidemiology and Human Trichinellosis

***Trichinella* spp in animals and humans of Southeast and South Asia: Status and gaps**

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Trichinella spp., has been reported in humans and animals across the globe including Southeast and South Asia, however, the systematically collected comprehensive information on the status of *Trichinella* spp. in humans, wild and domesticated animals of Southeast and South Asia is limited.

PubMed database was searched to retrieve information on *Trichinella* spp. in animals using key words “Trichin*” and “country’s name”, and studies from 2001 to 2023 were included for screening. *Trichinella* spp. infection (based on the artificial digestion) and/ exposure (based on serological methods) has been reported in animals (mostly pigs) in Thailand, Laos, Vietnam, India, Nepal, Philippines and Myanmar; dogs in Vietnam and Thailand were reported seropositive for *Trichinella* spp. Several outbreaks of trichinellosis have been recorded in Southeast Asia, especially in Thailand, Laos, Cambodia and Vietnam, and were linked to the consumption of raw, uncooked or fermented meat of pigs or wild boar. An outbreak occurred in India (a South Asian country) was associated with consumption of undercooked meat of wild boar. Of 13 species of *Trichinella*, *T. spiralis*, and *T. papuae* are prevalent in animals in Southeast and South Asian countries. Limited primary health care and diagnostic facilities could contribute to underreporting of foodborne infections including *Trichinella* spp. in the developing nations. The lack of human outbreaks in Bangladesh, Pakistan, Bhutan, Maldives, Brunei and Malaysia could be attributed to several factors including religious and dietary reasons. Inappropriate rearing practices and disposal of pig carcasses, lack of meat inspection and of biosecurity measures, and feeding of uncooked feed to pigs, could be the potential drivers for the persistence of *Trichinella* spp. infection in animals. There is a need to develop monitoring strategies and to adopt control measures to stop the transmission of this zoonotic nematodes in pigs of Southeast and South Asia.

2.2 Session II: Multidisciplinary, China

Extracellular vesicles from *Trichinella spiralis*: Proteomic analysis and protective immunity

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Abstract: *Trichinella spiralis* (*T. spiralis*) ML extracellular vesicles (EVs) are an important mechanism for the delivery of various functional molecules to host tissues and cells. In our study, we enriched Ts-ML-EVs by ultracentrifugation. TEM result showed that the morphology of isolated vesicles was consistent with classical parasite EVs. We first performed proteomic analysis of Ts-ML-EVs by LC-MS/MS. A total of 753 proteins were identified from Ts-ML-EVs, including several enzymes including serine protease, glutamate dehydrogenase and cytosol aminopeptidase. These proteins were enriched in different categories by GO, kyoto encyclopedia of genes and genomes (KEGG) and domain analysis. Among them, serine protease (SP) plays an important role in the interaction between *T. spiralis* and host. Our present study showed that mice injected subcutaneously with Ts-ML-EVs significantly reduced adult worms and ML burden, suggesting EVs contain antigens that can effectively reduce the transmission and pathology of *T. spiralis*. Cellular and humoral immune responses induced by Ts-ML-EVs were detected, including the levels of specific antibodies (IgG, IgM, IgE, IgG1 and IgG2a) as well as cytokines (IL-12, IFN- γ , IL-4 and IL-10) in serum. The results showed that Ts-ML-EVs could induce a Th1/Th2 mixed immune response with Th2 predominant.

This study revealed a potential role of Ts-ML-EVs in *T. spiralis* biology. A better understanding of EVs will be helpful in the interaction of parasite-host and development of *T. spiralis* vaccines.

Keywords: *Trichinella spiralis*; Extracellular vesicles; Th2

Session II: Multidisciplinary, China

The gut microbiota contributes to changes in the host immune response induced by *Trichinella spiralis*

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Abstract

The gut microbiota plays an important role in parasite-host interactions and the induction of immune defense. *Trichinella spiralis* is an important zoonotic parasite that can directly or indirectly interact with the host in the gut. Changes in the gut microbiota following infection with *T. spiralis* and the role of the gut microbiota in host immune defense against *T. spiralis* infection were investigated in our study. 16S rRNA sequencing analysis revealed that infection with *T. spiralis* can reduce the diversity of the gut microbiota and alter the structure of gut microbiota at early infection, which was restored when the worm left the gut. Antibiotic treatment (ABX) and fecal bacterial transplantation (FMT) were used to investigate the role of the gut microbiota in the host expulsion response during infection with *T. spiralis*.

We found that ABX mice have a greater burden of parasites, and the parasite burden decreases after fecal bacteria transplantation. The results of flow cytometry and qPCR revealed that disturbance of the gut microbiota affects the proportion of CD4⁺ T cells and the production of IL-4, which results in weakening of Th2 responses and makes expulsion difficult. In addition, as the inflammatory response decreased with the change in the microbiota, the Th1 response was also downregulated. The metabolomics results were also in good agreement with these findings, as the levels of inflammatory metabolites such as ceramides were reduced in the ABX group. In general, *T. spiralis* infection can cause changes in the gut microbiota, and the presence or absence of microbes can also weaken intestinal inflammation and the expulsion of *T. spiralis* by affecting the host immune response.

Keywords: *Trichinella spiralis*, gut microbiota, immune response, intestinal inflammation and pathology

Funding: This study was supported by the National Key Research and Development Program of China (2021YFC2600202), National Natural Science Foundation of China (NSFC32230104, 82201959) and the Natural Science Fund of Shanxi Province (20210302123297).

Session II: Multidisciplinary, China

β -Glucan-triggered *Akkermansia muciniphila* expansion facilitates the expulsion of intestinal helminth via TLR2 in mice

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Abstract

Trichinellosis caused by *Trichinella spiralis* is a serious zoonosis with a worldwide. β -Glucans (BG) are readily used across the world with noted health benefits, yet the effect and mechanism of BG on host defense against helminth infection remain poorly understood. We observed that BG could trigger worm expulsion via mucus layer independently of type 2 immunity, but was dependent on the gut microbiota in mice. BG restored the abundance of Bacteroidetes and Proteobacteria changed by *T. spiralis* infection to the control group level and markedly increased the relative abundance of Verrucomicrobia. *Akkermansia* (belonging to Verrucomicrobia) were significantly expanded in the BG + *T. spiralis* group. Notably, daily oral supplementation of pasteurized *A. muciniphila* has a stronger deworming effect than live bacteria and interacted with TLR2. These findings of this study is an easily implementable strategy to facilitate expulsion of gastrointestinal helminth.

Keywords: *Akkermansia muciniphila*; Microbiota; STAT6; TLR2; *Trichinella spiralis*; β -Glucan.

Session II: Multidisciplinary, China

The immunosuppressive ability of *Trichinella spiralis* adults can ameliorate type 2 inflammation in murine allergy model

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Abstract

Background: Recently, the incidence of allergic diseases is increasing year by year worldwide. The immunoregulation of *Trichinella spiralis* (*T. spiralis*) had great regulatory effect on alleviating immune-mediated inflammatory diseases. However, the stage at which *T. spiralis* exerts this immunomodulatory effect and its associated cellular regulatory network remains elusive.

Methods: BMDMs were stimulated with Excretory-secretory products of *T. spiralis*, and the differentiation were evaluated. Based on the OVA-induced murine allergic asthma model, *T. spiralis* was artificially infected at the three phases of allergy. The levels of cytokines, the cell subsets of macrophages, DCs, T cells in lung, and B cells, T cells in spleen, and intraperitoneal macrophages were evaluated.

Results: The mice infected *T. spiralis* showed the reduced inflammation of lung compared to OVA group. *T. spiralis* infection increased levels of anti-inflammatory cytokines (IL-10 and TGF- β 1) and decreased levels of Th2 cytokines (IL-4, IL-5 and IL-13) and alarms (IL-25, IL-33 and TSLP). The recruitment of eosinophils, CD11b⁺ DCs and interstitial macrophages to lung was significantly suppressed, but the proportion of Treg cells in spleen and alternative activated macrophages in peritoneal cavity was increased in *T. spiralis* infection groups compared with OVA group. Moreover, when *T. spiralis* infected prior to OVA-challenge, intestinal adults have ability to promote the proportion of CD103⁺ DCs and alveolar macrophages.

Conclusions: The results showed that *T. spiralis* had strong immunoregulatory ability to suppress type 2 inflammation, and adults had protective effect on the immune homeostasis in lung.

Keywords: allergic asthma, alternative activated macrophages, immunosuppressive response, *T. spiralis*

2.3 Session III: Phylogeny, Genomics and Proteomics

Keynote

Low-pass whole genome sequencing: a rapid means to trace the evolutionary history of *Trichinella spiralis*

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Abstract

Understanding the historical connections among parasite isolates can assist with identifying the sources of infection and tracing routes of transmission which benefits public health. Genetic information provides a powerful means to augment such investigations, but “molecular epidemiology” in parasitology lags viral and bacterial epidemiology. Longstanding barriers to timely analysis have relegated researchers to retrospective assessment of *Trichinella spiralis* transmission. A proof of principle analysis was conducted using *Trichinella spiralis* outbreak samples from the United States to demonstrate the utility of “low pass” whole genome sequencing in examining genetic relationships among isolates. For each isolate, 65 million base pairs of next generation sequencing reads were mapped to published *T. spiralis* chromosome sequences and genotypes were “imputed” based on quality scores of each base call. In phylogenetic comparisons with samples from Europe and Asia, genotypes from low pass sequencing formed highly supported clades for each outbreak. This technique requires only two days to assess possible transmission links among an array of samples contaminated with *T. spiralis*, yielding evolutionary trees capable of affirming or undermining suspected historical links. As databases of sequenced isolates increase in size, the method will become even more powerful as regional genetic signatures become more well defined.

Thus, low pass genome sequencing provides a new tool to trace the historical connections among *T. spiralis* isolates, especially when good reference genomes are available.

Session III: Phylogeny, Genomics and Proteomics

Long read sequencing vastly improves assembly of *Trichinella* genomes: updates and predictions

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Abstract

Genome sequences underpin marked advances in understanding the evolution and epidemiology of species of *Trichinella*. For example, over three hundred studies have cited the first publication of a genome in the genus, 12 years ago (Mitreva et al, 2011) and those data provided a basis for advances in molecular systematics, diagnostics, and population genetics. Draft genomes for all species then known in the genus (Korhonen et al, 2016) provided ever clearer understanding of what species of *Trichinella* share, and what features distinguish among them. Such information has proved useful in spite of the fact that such genome assemblies remain broken into hundreds or thousands of pieces, interrupted by highly-repetitive regions that defy easy and confident assembly. Long reads proved capable of improving assemblies ten-fold (Thompson, et al 2017), yielding evidence that genomes have maintained synteny over 7 millions of years of divergent evolution in species of the genus. Here, we report further progress in exploiting long-read sequencing techniques and improved assembly approaches to near complete, chromosomal-length genome assemblies (for *Trichinella spiralis*, *Trichinella nativa*, *Trichinella nelsoni*, and *Trichinella pseudospiralis*) and discuss biological insights related to stasis and change in the organization of *Trichinella* genomes, and discuss purposes to which such information can be deployed.

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

Keynote

Roles of *Trichinella*-secreted extracellular vesicles in the immune evasion and parasitism

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Extracellular vesicles (EVs) are heterogenous nano-sized vesicles secreted by nearly all kinds of cells. They participate in intercellular communication by carrying and transferring various kinds of biomolecules between cells, including proteins, nuclear acids, glycans and lipids. Cumulative evidence has revealed that EVs contribute to many aspects of physiological and pathogenic processes. The life cycle and parasitism of *Trichinella* are complicated, for example, intestinal infective larva (IIL) and adult worm (AW) in intestine involving in invasion of mucous membrane and regulation of worm expulsion, and muscle larva (ML) in muscle involving in nurse cell formation and long-term survival. It is generally considered that *Trichinella* takes many complicated strategies to manipulate host responses in its parasitism. Excretory-secretory (ES) products secreted by *Trichinella*, a mixture of many kinds of biomolecules including proteins are most likely to take part in these functions. Those biomolecules in ES are thought and investigated to be secreted from the parasites in a free form. However, recent studies suggested that the EVs may be a new vehicle to play roles in the immune evasion and parasitism during *Trichinella* infection. However, the whole picture and functions of *Trichinella*-secreted EVs are still obscure. Therefore, in the present study we identified the EVs from two main stages of *Trichinella spiralis*, AW and ML. We analyzed the specific, unique as well as common functions of the EVs. Firstly, we identified the EVs of *T. spiralis* as stage-specific in adult worms in host intestine (AW-EVs) and muscle larvae in host muscles (ML-EVs).

These stage-specific EVs exhibited unique or common functions, such as suppressing mucin production of intestinal cell line by AW-EVs and promoting differentiation of myoblast cell line by ML-EVs, and inhibiting IL-6 response of macrophages by both the EVs. Then, we carried out proteomics and whole microRNA sequencing analysis of the EVs, and found more than hundred kinds of proteins and microRNA in both the EVs. Furthermore, by analyzing the candidates with high reads or copies and stage-specific, we identified several EV-derived proteins and microRNA which may possess the functions in immunomodulation, immune evasion, worm expulsion and nurse cell formation. The results indicated that *Trichinella* communicates with host through its secreted EVs which contain stage-specific or common biomolecules to regulate the host microenvironment for immune evasion and parasitism.

Our study may provide new insight into the understanding of parasitism mechanisms in *Trichinella* infection.

Keyword: *Trichinella spiralis*, extracellular vesicle, excretory-secretory product, microRNA, parasitism

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

Keynote

Immunomodulatory potential of *Trichinella spiralis* antigens: possible solutions for inflammatory disorders

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Trichinella spiralis is a master of immunomodulation. This parasite manages to survive and complete its lifecycle in one host, protected from the host immune response inside the nurse cell. Communication with the host organism is achieved through excretory-secretory products released by encapsulated muscle larvae (ES L1). Through this complex mixture of proteins *T. spiralis* creates an environment dominated by anti-inflammatory and regulatory mechanisms responsible for restraining excessive inflammation and maintaining homeostasis. In this way, the parasite protects itself and the host organism at the same time. The infection with *T. spiralis*, as well as application of ES L1 products can alleviate or prevent the development of autoimmune, allergic and malignant diseases. Our research is focused on discovering the molecules and mechanisms underlying the protective effects of these products, with the ultimate goal of translating the knowledge gained from animal models into a therapeutic approach in humans.

Keywords: *Trichinella spiralis*, excretory-secretory products, immunomodulation

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

Do extracellular vesicles from *Trichinella spiralis* muscle larvae harbor the potential to induce regulatory T cells?

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During the chronic phase of the infection, *Trichinella spiralis*, through its excretory-secretory products (ES L1), induce Th2 and regulatory immune responses, thus creating nonpermissive environment for the development of autoimmune and other chronic inflammatory diseases. Our major goal is to discover immunomodulatory components within ES L1 products, so that we can characterize them and use them to design treatment for humans. Extracellular vesicles, isolated from *T. spiralis* ES L1 products (TsEVs), may be the important players in initiation, progression and regulation of the immune response, via their impact on dendritic cells. Human monocyte derived dendritic cells (DCs) were treated with TsEVs and subsequently co-cultivated with allogenic T cells. Phenotypes and cytokine production of DCs and T cells were determined by flow cytometry. TsEVs proved to be potent inducers of stable tolerogenic DC phenotype. TsEVs treated DCs express HLA-DR, CD-40, CD-86 almost at the level of the control, untreated DCs, expression of CD-83 was slightly elevated, while the expression of ILT-3 and CCR-7 was significantly increased compared to control. Elevated production of anti-inflammatory and regulatory cytokines IL-10 and TGF- β , alongside semi-mature phenotype indicates the capacity of these cells to polarize T cell immune response towards Th2 and regulatory type. Indeed, T cells co-cultured with TsEVs stimulated DCs showed significant increase in the production of IL-4 and IL-10 with the production of IFN- γ at the level of the control. Moreover, TsEVs stimulated DCs induced expansion of CD4⁺CD25⁺Foxp3⁺ regulatory T cells.

We can conclude that TsEVs act in a similar way as *T. spiralis* ES L1, in terms of inducing tolerogenic phenotype of DCs and regulatory response of T cells, which means that they have the capacity to convey the immunomodulatory properties of ES L1. Further studies will be focused on the possibility of using TsEVs as potential therapeutics for inflammatory disorders. (Funded by Ministry of Science, Technological Development and Innovation, Republic of Serbia, Co. No. 451-03-47/2023-01/200019)

Keywords: *Trichinella spiralis*, extracellular vesicles, regulatory T cells

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

Influence of the specific immune response to SARS-CoV-2 virus antigens on the immune response to *Trichinella spiralis* antigens and vice versa

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During an outbreak of trichinellosis in the village of Pozezeno, Veliko Gradiste, Serbia, specific anti-*Trichinella* antibodies were confirmed in 26 out of 28 persons suspected of having trichinellosis (National Reference Laboratory for Trichinellosis NRLT-INEP). The source of infection was a traditional meat product infected with *T. spiralis* (7 LPG, EURLP, ISS, Rome, It). The clinical picture of the patients was mild without complications and there were no inpatients. Fifteen out of 26 people infected with *T. spiralis* consented to participate in the study of monitoring the humoral and cellular immune response.

They were previously in contact with antigens of the SARS-CoV-2 virus either by natural infection (covid-19), vaccination (two or three doses of the vaccine) or both. Humoral immune response to *Trichinella* was determined by indirect immunofluorescence assay in the second serum sample (10 weeks p.i.) in one patient, while in the first sample the result was negative (6 weeks p.i.).

In 9 out of 15 patients, there was an increase in the *Trichinella* antibody titer in the second sample.

The presence of anti-RBD SARS-CoV-2 antibodies was detected by ELISA in 13 patients, while two patients were negative, and according to anamnestic data, they were not vaccinated, but recovered from covid-19 seven months earlier. In order to examine the mutual influence of the specific immune response to SARS-CoV-2 and to *T. spiralis*, the obtained results were compared with the immune response in 15 healthy persons as a matched control depending on age, vaccinated and/or recovered status regarding Covid-19. Memory B lymphocytes specific for *T. spiralis* and for SARS-CoV-2 were detected in all patients using flow cytometry. Analysis of CD4⁺ and CD8⁺ T lymphocytes showed that *Trichinella* infection did not affect the capacity of SARS-CoV-2-specific effector T cells to produce Th1-type cytokines IFN- γ , TNF- α and IL-2, so it can be concluded that *Trichinella* did not cause suppression of the type 1 immune response to virus antigens. On the other hand, effector T cells of trichinellosis patients responded to excretory-secretory *Trichinella* antigens by producing Th2-type cytokines, IL-4, IL-10 and IL-13 indicating that SARS-CoV-2 virus did not disrupt the induction of type 2 immune response. This study assessed for the first time the interplay between the *Trichinella*-specific immune response and the SARS-CoV-2 immune response and showed that *Trichinella* infection in humans suppresses neither antibody production nor a competent cellular response to SARS-CoV-2 virus antigens, and vice versa. (Funded by Ministry of Science, Technological Development and Innovation, Republic of Serbia, Co. No. 451-03-47/2023-01/200019)

Keywords: *Trichinella spiralis*, outbreak, immune response, SARS-CoV-2 virus

2.5 Session V: Detection, Legislation and Control

Keynote

Importance of food-borne parasites in a One health setting

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Background and aims. In 2012, WHO/FAO ranked 24 FBP using multicriteria decision analysis (MCDA) to provide risk assessors with a basis for prioritising control of highly ranked FBP on the global level. One conclusion was that ranking may differ substantially per region. In Europe, the same methodology was used to rank FBP of relevance for Europe. In Europe, FBP were ranked and surveillance systems of the five top prioritised FBP in the human and animal population evaluated.

Methods. Ranking was done using multicriteria decision analyses, surveillance and reporting systems for both human and animal populations were analysed for five different European regions for the highest-ranked FBP in Europe, all of which are zoonotic.

Results. For most FBP, human surveillance is passive in most countries and regions in Europe and notification differs between countries and regions. There is a lack of adequate surveillance programmes for these FBP, except for *Trichinella spp.*, notifiable in 34 countries with active surveillance in susceptible animals under EU directive in the member states.

Conclusions. Human and animal surveillance are present for the top five prioritised FBP but substantial underreporting and a lack of consistency in surveillance and reporting requirements exist between European countries. This may hamper the true disease burden of foodborne parasites in Europe. New initiatives to get more insight will be discussed.

Reference: van der Giessen J, Deksne G, Gómez-Morales MA, Troell K, Gomes J, Sotiraki S, Rozycki M, Kucsera I, Djurković-Djaković O, Robertson LJ. Surveillance of foodborne parasitic diseases in Europe in a One Health approach. *Parasite Epidemiol Control*. 2021 Feb 3;13:e00205. doi: 10.1016/j.parepi.2021.e00205. PMID: 33665388; PMCID: PMC7900597.

Session V: Detection, Legislation and Control

Understanding the Global Connection: investigating the association between pork meat exports and trichinellosis

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The presence of trichinellosis in a country can result in trade restrictions and product blockages due to safety concerns. This scenario is exemplified by the case of "Mad Cow Disease" (Bovine spongiform encephalopathy), where fears of endemic diseases hindered trade between nations. Such endemic fears often lead to trade barriers and impede economic exchanges, underscoring the importance of effective prevention and control strategies to maintain market access and safeguard public health. The aim of the present study was to explore the relation between the volume of swine meat exports and reported trichinellosis cases. To this end, we compared the volume of exported pork meat with the reported cases of trichinellosis in European and two South American countries. The equation $\text{Expo}(i,t) = \beta_0 + \beta_1 T(i,t) + \beta_3 X(i,t) + \varepsilon(i,t)$ represents the model, where $\text{Expo}(i,t)$ denotes the volume of exports for country i in year t , $T(i,t)$ indicates the number of reported trichinellosis cases for country i in year t , $X(i,t)$ represents a set of control variables, and ε refers to the idiosyncratic error term. Data on the volume of swine meat exports during 2015-2019 were sourced from Eurostat (Europe), INDEC (Argentina), and Odepa (Office of Agricultural Studies and Policies, Chile). Data from the Health Ministries of Argentina and Chile, as well as from the European Centre for Disease Prevention and Control (ECDC) were consulted for information on *Trichinella* human cases.

Data processing and analysis were conducted using Stata 17. The study reveals a negative correlation between the variables in several countries with a substantial number of cases, providing valuable insights into the relationship between the volume of pork meat exports and reported cases of trichinellosis. Given those findings, it is imperative to conduct further investigations to explore the potential causality of trichinellosis within the framework of global trade. In conclusion, this study aims to underscore the adverse impact of trichinellosis on pork meat trade. Therefore, endemic countries should prioritize comprehensive prevention and control strategies to mitigate the detrimental effects on pork meat commercialization and ensure public health protection.

Keywords: trichinellosis, trade restrictions, pork meat, export

This work was supported by Universidad de Buenos Aires, Secretaría de Ciencia y Técnica Subsidio UBACyT 20020190200396 BA and UBACyT 20020170100530BA and Ministerio de Ciencia y Tecnología, FONCyT Subsidio PICT-2018- 01203.

Session V: Detection, Legislation and Control

Laboratories for *Trichinella* testing: Concept of surveillance in Lower Saxony and insights from the audits

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EU legislation establishes special rules for the control of trichinellosis, including the requirement for systematic tests for *Trichinella* in all slaughtered pigs, wild boar and solipeds. Domestic swine may be exempt from *Trichinella* examination if the animals come from officially recognised controlled housing conditions. In Germany, the predominantly part of slaughtered domestic swine is tested for trichinella.

Official laboratories with sole activity in the detection of *Trichinella* in meat by applying the methods laid down in Regulation (EU) No. 2015/1375 may be designated by competent authorities in accordance with Regulation (EU) No. 2017/625, even if they are not accredited. In the Regulation, it is specified that these laboratories carry out *Trichinella* examinations under the supervision of the competent authority or an official laboratory accredited according to the EN ISO/ IEC 17025 standard for the detection of *Trichinella*.

In Lower Saxony, there are in total 58 laboratories for *Trichinella* testing (TUS), 56 do not have an accreditation. 17 TUS are located in slaughterhouses, 20 in local veterinary offices and 19 in veterinary practices or in laboratories connected to the private premises of official staff.

Two TUS are located within external accredited laboratories, one of which is located in the Food and Veterinary Institute Braunschweig/Hannover, Hannover site (VI-H). The VI-H is accredited for the detection of *Trichinella* and is supervising 40 TUS.

Since 2020, all TUS under supervision of VI-H have been visited at least twice. The focus of the audits carried out in the TUS is on checking the equipment (by means of calibrated measurement standards), the documentation, traceability and qualification of staff as well as assessing the work processes.

The most frequent deviations found related to the use of non-up-to-date versions of the QMS and insufficient documentation. The sieves were frequently found to be in an inadequate state of cleaning. Four balances, five microscopes and two magnetic stirrers were replaced because they did not meet the requirements of ISO 18743 or due to intolerable deviations found during the checks. In one case, the cancellation of the designation by the competent authority was requested due to failure in the inter-laboratory comparative test.

Recently, laboratories under supervision of VI-H are audited in a risk-based approach. The risk assessment is carried out according to defined characteristics and is the basis for determining the control frequency for the next scheduled on-site visit. A maximum inspection frequency of three years can be achieved. In 2023 a one-year and two-year frequency was set for half of the TUS respectively.

Session V: Detection, Legislation and Control

Analysis of historical data for *Trichinella* proficiency testing: towards the definition of performance evaluation standards for routine laboratories

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In France, as in many EU and non-EU countries, meat inspection is carried out by decentralized laboratories (departmental veterinary analysis laboratories (LVD)), usually closed to slaughterhouses. These LVDs are an essential link in the food safety chain and form a network led by a national reference laboratory (NRL). Among its missions, the NRL must ensure that LVD perform reliable analysis in accordance with the regulation and that their results are in line with expectations. The official analysis for *Trichinella* detection in meat benefits for some years from ISO 18743 as the international standard for the method to be used by veterinary services. This ISO standard is derived from the gold reference detection method published in the EU regulation 2015/1375 and previously in EU 2075/2005.

In order to control the effectiveness of the tests in France, the NRL has implemented a national proficiency testing since 2004. The NRL prepares proficiency samples consisting of pork meat spiked with a known number of *Trichinella* muscle larvae inside their collagen capsule. The LVDs then receive a set of these samples each year, analyze them according to the official method, count the larvae for each sample and return the count results to the NRL for performance evaluation.

Today, there are no data processing tools and therefore no gold standard to analyze results and assess the performance of laboratories. We propose a statistical analysis of the data accumulated since 2004.

The feedback on these accumulated data is therefore important and allows to establish a reference that would be a kind of princeps in the field of Quality Assurance of *Trichinella* diagnosis. The following points will be presented: laboratory effect, year effect, evaluation of laboratories with designation of an assigned value and standard deviation, monitoring of individual laboratory performance.

Session V: Detection, Legislation and Control

***Trichinella patagoniensis* isolation from naturally infected puma from Neuquén province, Argentina**

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For many years, *T. spiralis* was the only species detected in Argentina. However, since 2004, it has been documented the presence of the encapsulated *Trichinella* species *T. patagoniensis* and *T. britovi* as well as the unencapsulated *T. pseudospiralis* in domestic and wild animals. The aim of the present research is to describe the first record of *Trichinella patagoniensis* in a cougar (*Puma concolor*) from the province of Neuquén, Argentina. A sample of 80 g of diaphragmatic muscle tissue from a male cougar found dead from the locality of Aluminé (38.9425° S, 70.9369° O) in Neuquén Province, Argentina, was analyzed using the artificial digestion technique. Larvae were preserved in 96% ethanol. To identify the *Trichinella* species at the molecular level, DNA was extracted from five individual muscle larvae (ML) using the DNeasy Blood & Tissue Kit (Qiagen®). For species identification, 10 ng of DNA was used for PCR amplification of the expansion segment V (ESV). Additionally, a fragment of 311 bp of the mitochondrial cytochrome C oxidase subunit I (COI) gene was amplified by PCR, sequenced bidirectionally and compared against the COI sequences of *Trichinella* spp. available in GenBank. ML with morphological features compatible with the *Trichinella* genus were recovered with a larval burden of 163 larvae per 80 grams of tissue, corresponding to a density of 2.03 larvae per gram of tissue.

The multiplex PCR performed on individual ML produced single bands resembling those of *T. nativa*, *T. britovi*, *T. murrelli*, *Trichinella* T6, and *T. chanchalensis*, which was not useful for diagnosing *Trichinella* T12. The obtained COI sequence showed highest percent identity (99 to 100 %) with the isolates of *Trichinella patagoniensis*. This study presents the first report of *Trichinella patagoniensis* circulating in the province of Neuquen, Argentina, and adds to the limited knowledge of this parasite in the country. Our findings also mark the fourth time that an isolation of *Trichinella patagoniensis* has been reported in cougars from Argentina, highlighting the potential public health and wildlife conservation implications of this zoonotic pathogen.

Keywords: *Trichinella patagoniensis* - cougar - Neuquen – Argentina

This work was supported by Universidad de Buenos Aires, Secretaría de Ciencia y Técnica Subsidio UBACyT 20020190200396 BA and UBACyT 20020170100530BA and Ministerio de Ciencia y Tecnología, FONCyT Subsidio PICT-2018- 01203.

Session V: Detection, Legislation and Control

***Trichinella britovi* and *T. spiralis* infections in large carnivores and other wild species in Switzerland**

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Large carnivores including wolves (*Canis lupus*), golden jackals (*Canis aureus*) and lynx (*Lynx lynx*) are officially monitored species in Switzerland. Deceased individuals are subjected to post-mortem examination and collection of baseline health data. The procedure includes an assessment of different infectious agents, including *Trichinella* spp., the cause of a notifiable zoonotic infection. Between May 2009 and May 2023, a total of 354 large carnivores were tested at the National Reference Laboratory for Trichinellosis by the artificial digestion method. Besides, muscle samples from 8,839 wild boars (*Sus scrofa*), 27 red foxes (*Vulpes vulpes*), and 23 European badgers (*Meles meles*), mainly submitted by Swiss hunters, were also analysed for *Trichinella* infection. *Trichinella* spp. were detected in 16/100 (16%) wolves, 1/4 (25%) golden jackals, 41/250 (16.4%) lynx, 2/27 (7.4%) red foxes, 0/23 (0%) badgers, and 2/8,839 (0.02%) wild boars. All positive cases were further tested by multiplex PCR to identify the parasite at the species/genotype level. Two different species were detected: *T. britovi* (in 14 wolves, 1 golden jackal, 33 lynx, 2 red foxes and 1 wild boar) and *T. spiralis* (in one lynx and one wild boar). In nine cases, the molecular identification was not conclusive. All positive cases were autochthonous, except for the wild boar with *T. spiralis* infection, which had been hunted in France and imported into Switzerland. Although no cases of *Trichinella* spp. infection in domestic swine and horses were detected in the last decades in Switzerland, we revealed that these parasites, most frequently *T. britovi*, are still present in Swiss wildlife.

Therefore, the occurrence of sporadic infection in domestic animals cannot be excluded.

Session V: Detection, Legislation and Control

Quantitative microbial risk analysis is a useful mathematical tool to evaluate relative risks regarding foodborne parasites

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Abstract: Domestic pigs and wild boar are the main sources of human infection in Europe and meat of these animals is tested for *Trichinella* at slaughter to prevent disease, following EU Regulation 2015/1375 (European Commission, 2015) and NEN-EN-ISO standard 18743:2015 (European Committee for Standardization, 2015), which describe the method for detection of *Trichinella* muscle larvae in muscle tissue at carcass control.

Recently, a quantitative farm-to-fork risk model has been developed for *Trichinella* species in pork and wild boar, that included infection status of the animal, the process of sampling, test sensitivity at carcass control, partitioning of edible pork parts, *Trichinella* larva distribution in different muscle types, heat inactivation at cooking and consumption portion size. Later, this model was completed by including different housing types for pigs: controlled (bio-secure) housing versus non-controlled housing. Finally, the inactivation of *Trichinella* larvae at home-cooking was evaluated in more detail.

These examples show how quantitative microbial risk analysis can be used to evaluate relative risks in the chain from farm to fork.

Keywords: *Trichinella*, quantification, dose response, risk evaluation

Session V: Detection, Legislation and Control

Pork Quality Assurance in the United States: evidence of negligible risk.

Benjamin Rosenthal and Valsin Fournet

USDA-Agricultural Research Center

Pork Quality Assurance Plus (PQA Plus) is an education and certification program designed to help pig farmers and their employees continually improve production practices, enhancing food safety, animal well-being, environmental stewardship, worker safety, public health and community. Individuals can become certified through an education program, and farms can receive PQA Plus site status through an on-farm site assessment with a PQA Plus Advisor. Here, we report progress toward completing an ambitious survey of swine raised under this certification, using the artificial digestion method, to assess whether this production compartment constitutes one of negligible risk. To date, no evidence of infection has yet surfaced in any of over 2 million tested animals.

Plenary

Trichinella; becoming a parasite

Dante Zarlenga (Retired)

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Within the Nematoda, most agree that free-living nematodes were the ancestors of parasitic nematodes. Data are also consistent with parasitism among nematodes having evolved independently at least 15 times. Thus, searching for the multitude of genes and ascertaining the number of genetic changes that coincide with character transformation to parasitism has been enigmatic because the high level of genetic and biological diversity among parasites dictates an equally high level of diversity in the transition to parasitism and the adaptive process. However, examining holistic mechanisms such as horizontal gene transfer (HGT) has shed light on the acquisition and maintenance of important characters among all walks of life and more recently in the evolution of plant parasitism. We hypothesize that HGT played an equally important role in the evolution of parasitism among early ancestors of *Trichinella*. However, transitioning to parasitism tells only one part of the story. How a parasite adapts to new environments amidst our ever-changing climate is of equal importance to worm longevity and can be explained in part by the Stockholm Paradigm that helps clarify the conflict between cospeciation and colonization of a host. The Stockholm Paradigm amalgamates 4 core principles; Ecological Fitting (host switching followed by evolution), the Oscillation Hypothesis (a generalist parasite becomes a specialist), the Geographic Mosaic Theory of Coevolution (co-adaptive processes), and the Taxon Pulse Hypothesis (vacillating between biotic expansion and isolation). We hope to briefly investigate how one or more of these interactive theories may explain the varying levels of diversity among *Trichinella* genotypes.

Keywords: Horizontal gene transfer, cyanase, evolution, parasitism

3 Abstract for Student Research Award

Abstract for Student Research Award

***Trichinella spiralis* E3 ubiquitin ligase *Ts*-RNF ubiquitylates SQSTM1/p62 and inhibits myoblast differentiation to participate in cyst formation**

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Abstract: *Trichinella spiralis* nematode infections can form cysts in host skeletal muscle cells, allowing the nematodes to evade host immune responses and maintain growth and development. Notably, pathogen infections can interfere with and exploit the ubiquitin–proteasome pathway to allow the pathogens to evade host defenses. In our study, the modification sites of the substrate were screened and validated using ubiquitination modification quantitative proteomics.

The first identified *Trichinella spiralis* ubiquitin ligase *Ts*-RNF was found to interact with SQSTM1/P62(sequestosome 1) and to modify UBA domain K422 by K63-linkage ubiquitination. Moreover, *Ts*-RNF was found to interfere with the host ubiquitin modification pathway and to affect autophagic flux, leading to impaired mitochondrial clearance during myoblast differentiation, abnormal myotube differentiation and fusion. In particular, interference with the *Ts*-RNF gene in vivo was able to affect the growth and development of *Trichinella spiralis* and even lead to death of the pathogen. Thus, these findings are the first to demonstrate that nematodes can interfere with host ubiquitination modification, providing new evidence for the mechanism of cyst formation in *Trichinella spiralis*.

It improves the understanding of parasite invasion and disruption of tissue homeostasis, and has important biological significance for the study of the relationship between host protein modification and parasite infection.

Keywords: *Trichinella spiralis*; E3 ubiquitin ligase; myoblast differentiation; cyst formation

4 Abstracts for poster presentations

4.1 Session I: Epidemiology and Human Trichinellosis

Surveillance in terrestrial and marine wildlife of Patagonia

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Trichinellosis is endemic in Argentina and an important public health problem because of its high morbidity rates. In order to know the regional epidemiology of *Trichinella*, it is important to maintain continuous surveillance that includes direct detection in wild species. The aim of this study was to analyze by artificial digestion skeletal muscle of terrestrial and marine vertebrates found dead by natural or anthropic action (run over, entangled in gillnets) in the east of northern Argentine Patagonia. So, between February 2020 and March 2023, skeletal muscles were collected from 42 animals. From them, 25 terrestrial: 12 Pampa Fox (*Lycalopex gymnocercus*), 3 Geoffroy's Cat (*Leopardus geoffroyi*), 1 Cougar (*Puma concolor*), 5 Little Grison (*Galictis cuja*), 2 Large Hairy Armadillo (*Chaetophractus villosus*), 1 White-eared Opossum (*Didelphis albiventris*), 1 Molina's Hog-nosed Skunk (*Conepatus chinga*); and 17 marines: 4 South American Sea Lion (*Otaria flavescens*), 1 Short-beaked Common dolphin (*Delphinus delphis*), 5 Franciscana Dolphin (*Pontoporia blainvillei*), 7 Magellanic Penguin (*Spheniscus magellanicus*). The muscle samples were preserved at 2-8°C. To test 31 animals, 100 grams free of indigestible fat, tendons, fascia, etc. were used. Of the other 11 animals, between 20 and 50 grams free of indigestible fat, tendons, fascia, etc. were tested.

Foreleg muscles were used in terrestrial mammals, axial muscles in marine mammals, and pectoral muscles in penguins. All artificial digestions were negative for the presence of *Trichinella* larvae.

Although the records of *Trichinella* in wild species (not for human consumption) are few in Patagonia, in a context of global change it is essential to carry out surveillance studies of this kind. Also in a region that has pig farms with insufficient biosecurity. Finally, the negative results provide valuable information for the construction of prevalences in *Trichinella* spp. cycle.

Keywords: *Trichinella*, artificial digestion, Argentina

Session I: Epidemiology and Human Trichinellosis

***Trichinella* (Nematoda) in South Africa, the possibility of a mesocarnivore (Mongoose) playing a role in the sylvatic cycle**

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Trichinella genus includes several species of important parasitic nematodes with zoonotic and One Health importance. Although some species have a cosmopolitan distribution, there is still much unknown in species/genotypes distribution on a global scale and their specific genetic features and relationships inside the genus.

South Africa is renowned for its exceptional biodiversity, which provides a wide range of hosts for different groups of parasites. So far, few *Trichinella* species have been reported in various hosts (African civet, lion, black-backed jackal, crocodile, multimammate mouse, spotted hyena), however, there is still much is unknown about the life cycle and diversity of host species, especially in the sylvatic cycle.

During 2016-2018, a study was conducted to investigate the presence of *Trichinella* larvae in various carnivorous mammals and birds. A total of 248 mostly roadkill specimens were sampled to be examined for the presence of *Trichinella* spp. larvae using the artificial digestion method and obtained larvae were analysed through molecular (PCR) methods.

Of the 248 examined samples, *Trichinella* larvae were detected in six individuals of five different animal species, i.e. Bat-eared fox, Black-backed jackal, Marsh mongoose, stray cat, and White-browed coucal.

Although the prevalence of infection was relatively low (2.42 %), our research provides deeper insight into the life cycle of *Trichinella* spp. in different African animal hosts. Newly identified host species emphasize the importance of further investigation, also taking into account the One Health concept, in particular in the context of national reference labs.

The analysis of five sequences of ESV locus using cluster analysis revealed that three samples, one from *Atilax paludinosus* and two from *Canis mesomelas*, were grouped in a single cluster. In contrast, samples from *Felis catus* and *Otocyon megalotis* were situated outside this cluster. A BLAST search in the GenBank database indicated that all sequences from the group were a match for *Trichinella britovi*, with the other two samples being *Trichinella spiralis*. Molecular analyses employing a different pair of primers used to differentiate *T. britovi* and T8 are currently underway and the results will be discussed.

However, the identification of a positive Marsh mongoose (*Atilax paludinosus*) represents the first report of this mesocarnivore host species expanding the known host range of this important parasite species and emphasizing the sylvatic cycle and the role of wildlife in parasite circulation and transmission.

Keywords: *Trichinella*, wildlife, zoonosis, PCR methods, *Atilax paludinosus*

Acknowledgement: The study was funded by the VEGA project No. 2/0014/21 and by the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under project No. 09I03-03-V01-00046.

Session I: Epidemiology and Human Trichinellosis

***Trichinella* spp. in Germany – current situation in wildlife**

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In Germany, *Trichinella* (*T.*) spp. are mainly detected in the sylvatic cycle. The affected animal species are wild boar, fox, badger, raccoon dog, wolf and raccoon. Detections in these animals are predominantly of the species *T. spiralis*, followed by *T. pseudospiralis* and *T. britovi*.

According to Regulation (EU) No. 2015/1375 official *Trichinella* inspection is mandatory for wild boars and other susceptible animal species if their meat is intended for human consumption.

In recent years, an increase in the number of *Trichinella*-positive wild boar hunted in Germany has been registered. For example, while in 2013 only 11 animals out of 443,214 investigated wild boars were *Trichinella*-positive throughout Germany, in 2018 this number had practically doubled. 22 animals were positive (out of a total of 454,136 animals). The prevalence of positive wild boar increased throughout Germany from 0.002% to 0.007% between 2012 and 2021.

However, the detection rate in wild boar shows clear regional differences. For example, in recent years, an increased number of *Trichinella* detections in wild boar has been registered in the eastern part of Germany. Here, the federal states Western-Pomerania, Brandenburg and Saxony that are geographically located on the eastern border are particularly affected. For example, in 2020, all positive wild boar hunted in Germany (32 animals) came from these bordering federal states; in 2021, 37 animals out of a total of 40 positive animals came from this region.

Parallel to the increase in positive wild boar, spread of raccoon dogs and wolves can be observed in these regions.

Since both animal species are susceptible to *Trichinella* spp. and *Trichinella*-positive raccoon dogs and wolves have already been detected, the importance of these animal contributing to the spread of *Trichinella* spp. is discussed.

The fact that there is currently an increase in *Trichinella* prevalence in certain regions indicates that proper disposal of carcasses is essential to break the *Trichinella* life cycle to prevent further spread. There is an urgent need to raise awareness among hunters in this regard. In addition, hunters should be made aware that a proper *Trichinella* examination is essential for any hunted animal susceptible to *Trichinella* spp. if the meat of that animal is intended for human consumption.

The current *Trichinella* situation concerning the prevalence in susceptible wild animals in Germany with regard to geographical distribution and developmental trends in recent years will be presented in form of a poster presentation.

Session I: Epidemiology and Human Trichinellosis

***Trichinella* spp. findings in European badgers in the Czech Republic**

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Trichinellosis is a food borne zoonotic disease caused by the consumption of raw meat and raw meat-derived products from animals infected with nematode larvae of the genus *Trichinella*. EU legislation (Regulation (EC) No. 2015/1375) establishes special rules for the control of trichinellosis including the requirement for systematic tests for *Trichinella* in all slaughtered pigs, wild boar and horses, except in pigs from holdings or compartments officially recognised as applying controlled housing conditions. Regular monitoring of foxes or other indicator wildlife animals is an important tool for assessing changes in disease prevalence. No positive *Trichinella* findings have been reported in Czech fattening and breeding pigs for over decades. *Trichinella* spp. circulate predominantly within the sylvatic cycle in wildlife and wild boars and foxes were found positive for *Trichinella* in the Czech Republic during regular systematic testing.

The European badger, *Meles meles* (Carnivora, Mustelidae), is an opportunistic omnivore that can be found throughout the Czech Republic with an estimated population of ca. 30,000 individuals. Number of badgers tested for *Trichinella* in the Czech Republic has increased during the last two decades. Overall, 3,064 badgers were examined by artificial digestion between 2003 and 2022. For species determination, larvae recovered from the artificial digestion were used for DNA isolation, and further processed by multiplex PCR. In total, three badgers (0.098 %) were found positive for *Trichinella* and two cases of *T. britovi* and one case of *T. spiralis* were confirmed.

The European badger hosted considerably higher *Trichinella* prevalence than in wild boars and is comparable to *Trichinella* prevalence in foxes tested in the Czech Republic, and should be considered as a sentinel species for surveys for *Trichinella* epidemiology. The consumption of European badger meat is relatively widespread in Czech hunter community. Therefore, programs are needed to emphasize the necessity to ensure testing for *Trichinella* spp. infection in European badgers intended for human consumption and promoting education of hunters regarding thorough cooking of badger meat to guarantee food safety.

Keywords: European badger, *Meles meles*, sentinel host species, prevalence, *Trichinella* spp.

Session I: Epidemiology and Human Trichinellosis

Title: First detection of *Trichinella nativa* in foxes (*Vulpes vulpes*) in Slovenia

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Abstract:

Different species of *Trichinella* infest birds, reptiles and various mammals, including humans. The feature of *Trichinella* is that they first infest the intestine and then the skeletal muscles of the host. The epidemiology of trichinellosis is very complex because there are different species of *Trichinella*, a large number of potential hosts, and different life cycles. Sylvatic cycle is independent of humans as hosts and is associated with wildlife populations where trichinella is transmitted primarily among carnivores via prey and carrion. *T. spiralis* is transmitted in the synanthropic cycle, in which the causative agents circulate within the pig population. Pigs get infested with insufficiently heat-treated garbage and animal waste products, ingestion of pig carcasses, and probably also by biting tails and ears. Wild animals, e.g., foxes and rats, can also be a source of infection for pigs. Elimination of these animals from the pig environment has been shown to help break the life cycle.

In the present study, the prevalence of *Trichinella* spp. in foxes during the 2020/2021 hunting season in Slovenia was investigated, providing valuable data for assessing the risk of trichinellosis transmission to other animals and humans.

A total of 680 foxes were examined and muscle samples from the lower forelimbs were tested for *Trichinella* larvae. A prevalence of 0.882% was found for *Trichinella* spp. and the two species identified were *T. britovi* and *T. nativa* with prevalence of 0.735% and 0.147%, respectively. This study is the first detection of *T. nativa* in Slovenia.

The discovery of *T. nativa* in foxes in Slovenia underscores the need for ongoing surveillance and control measures to limit the transmission of trichinellosis from wildlife to other animals and humans, taking into account the freeze resistance of *T. nativa* larvae. Proposed measures include education campaigns on safe meat handling and preparation, of meat, monitoring and control of wildlife populations.

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Session I: Epidemiology and Human Trichinellosis

***Trichinella* infection in two populations of golden jackal (*Canis aureus*) in Croatia**

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The territory of Croatia supports two populations of golden jackals separated both spatially and temporally. The older population has inhabited the islands and coastal parts of Croatia since the Middle Ages (Dalmatian population, DP). The second population of golden jackals is of recent origin, migrating in the 1990s from eastern Bulgaria and entering Croatia from the neighbouring countries (South-East European Population, SEEP).

These two populations in Croatia are separated by the Dinarides mountain range, home to the three apex predators (bear, wolf, and lynx) from which the golden jackal retreats, and so far there is no evidence of any mixture between these two populations in Croatia.

The golden jackal is a particularly important animal species in the epidemiological sense, as it has been proven to harbour numerous pathogens that cause zoonoses. The biological traits of the species enables its dominance in relation to other carnivorous and omnivorous animal species in most of Croatia where the apex predators are absent, particularly when feeding on organic waste in the wild but also in the vicinity of human settlements.

A study of trichinella infections in the golden jackal populations in Croatia was ongoing from 2008 to 2022. During this 15-year period, 186 samples were tested and infection was confirmed in 47 individuals (25.3%). *Trichinella spiralis* was identified as the dominant species (28 samples; 59.6%), followed by *T. britovi* in 13 samples (27.7%) (PCR failed in six samples). Infected golden jackals were found in nine of ten tested counties. Nearly equal infection rates with *Trichinella* parasites were found in both populations: 22.9% in DP, and 26.7% in SEEP. *T. spiralis* was identified in both populations, as a species typical for the domestic cycle, though with significantly different ratios: in DP the ratio of identified species was 10:6 in favour of *T. britovi*, while in SEEP the ratio was 22:3 in favour of *T. spiralis*. The obtained results were processed statistically, and the origin of the tested, positive, and identified samples was shown on the geographical map of Croatia. According to these findings, we conclude that the two golden jackal populations are an extremely important reservoir for parasites from the genus *Trichinella* in Croatia, for both the sylvatic and domestic cycles. Accordingly, there is a strong need for epidemiological surveillance for both populations.

Keywords: *Trichinella* infection, golden jackal, epidemiology, *T. spiralis*, *T. britovi*, Croatia

Session I: Epidemiology and Human Trichinellosis

About the spread of trichinellosis in Georgia in the 21st century

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Keywords: *Trichinella*, disease, healthcare, human trichinellosis

Among parasitic diseases, trichinellosis remains a significant healthcare challenge for years in Georgia. The first data about human infestation by trichinella was mentioned by Kalius (1946), who described the occurrences of the disease in Western Georgia in 1886-1892. The first epidemiological confirmed case was recorded in 1952. Until the 80s, only singular occurrences were registered. In 1980-1990 and later years, number of human diseases were increasing significantly (1980-1990s – 738; 1991-2000s - 1508). However, since 2010 the number of cases began to decrease.

Trichinella was registered in 14 species of mammals in Georgia. A high frequency of infestations was mentioned in stone martens (40.6%), jackals (36.6%), foxes (22.2%), wood mice (2.4%). Among domestic carnivorous animals, this helminth was recorded in cats (3.7%).

A retrospective study was conducted to assess the spread of human trichinellosis in Georgia. Within the framework of the study, all patients with trichinellosis, referred to the Tropical Medicine Research Institute of Medical Parasitology in 2017-2022, were studied and analyzed.

A total of 12 patients were registered in the mentioned period. In all cases, the diagnosis of trichinellosis was established through clinical-epidemiological findings, and the detection of anti-trichinella antibodies (ELISA) was used for laboratory confirmation.

These patients presented with: hectic fever (75%), myalgia (92%), periorbital oedema (83%), eosinophilia (92%). 42% of patients were treated as an outpatient, and 58% were hospitalized. No lethal outcome was observed. All patients mentioned the consumption of undercooked domestic pork, therefore in all cases, the source of infection was a domestic pig.

According to the data of the veterinary laboratory of the Ministry of Rural Agriculture, there is also a decrease in the rate of infection of pigs with trichinella, in 1996-2005 the mentioned rate was 3.7%, in 2006-2009 - 1.2%, and in 2019-2022 - 0.1%.

In 2008-2009, infestation with trichinella was revealed in predatory animals (fox, wildcat, wolf, pine marten) in Eastern Georgia (Akhmeta and Tianeti). The mentioned areas can be considered natural foci of trichinellosis of carnivorous mammals.

In recent years a reliable trend of reduction of trichinellosis in humans is expressed in Georgia. The course of disease is mainly mild or moderate, severe forms and lethal outcomes are not recorded. Although the infestation rate of pigs is reduced, the presence of *Trichinella* in wild animals indicates that Georgia is still under potential risk of the spread of trichinellosis, and preventative measures and systematic monitoring should be carried out.

Session I: Epidemiology and Human Trichinellosis

***Trichinella* infections in animals and humans in Italy from 2019 to 2022**

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In Italy, nematodes of the genus *Trichinella* circulate among wild susceptible animals confined to mountain areas, where foxes and wolves are the main reservoir hosts, since they have less access to food resources of human origin, and consequently, more likely they feed on carcasses of infected animals. The aim of this study was to gather the epidemiological data on the presence of *Trichinella* spp. in animals and humans in Italy from 2019 to 2022. During this period, an average of 9.5 million pigs were slaughtered and tested for *Trichinella* infections per year. A total of seven free-ranging pigs tested positive, 2 heads in 2020 and 5 in 2022 (2 in Tuscany region, Central Italy and 3 in Friuli Venezia Giulia region, Northern Italy); with a total prevalence of < 0.001% per year. In wildlife, the prevalence varied from 0.02% to 0.006% in wild boar (*Sus scrofa*), 0.2% - 0.7% in foxes (*Vulpes vulpes*) and 6% - 14% in wolves (*Canis lupus*). The etiological agents were *Trichinella britovi* in mammals and *Trichinella pseudospiralis* in a red kite (*Milvus milvus*). *Trichinella spiralis* was not documented in Italy in the period in question. Regarding human cases, at the turn of 2019-2020, a human outbreak of trichinellosis involving 88 persons (three hospitalized), was documented in the Piedmont Region (Northern Italy) due to the consumption of salami made with wild boar meat infected by *T. britovi*. In 2021, no case of trichinellosis was documented in Italy. In 2022, an outbreak of trichinellosis was documented in the Sardinia Island. One (the index case) of the 4 infected persons was hospitalized with severe myocarditis. The epidemiological investigations carried out by the veterinary services strongly suggested the consumption of *Trichinella* infected meat from wild boar or from free ranging pigs as the source of infection, but no meat samples were traced back.

In Italy, the presence of the sylvatic cycle of *Trichinella* spp. with the recurrence of outbreaks of trichinellosis caused by the consumption of meat from hunted wild boars or much less often from pigs illegally reared in the wild, indicates the need to periodically provide for awareness and education campaigns for hunters and people rearing backyard and free-ranging pigs on the risk of *Trichinella* spp. infection in wildlife.

Keywords: *Trichinella*, epidemiology, Italy, wildlife, surveillance

Session I: Epidemiology and Human Trichinellosis

Trichinella species infection in Romanian wildlife, a review

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Romania is a southeastern European country located in the north of the Balkan Peninsula. Nematodes of the genus *Trichinella* are important zoonotic parasites present throughout Romania. In Romania, the first information regarding trichinellosis dates back to 1866. A literature review of the past 30 years (original studies) concerning the only two species (out of the four in Europe) of *Trichinella* (*T. spiralis* and *T. britovi*) confirmed in wildlife from Romania was conducted and corroborated with the results of our original research concerning the topic. Based on the articles introduced in the current review, in Romania, European minks were infected with *T. spiralis*, while wolves, European wild cats, Eurasian lynx, golden jackals, stone marten, and European badgers were infected with *T. britovi*, respectively. Both *Trichinella* species have been identified in foxes, bears, wild boars, and ermines, but recently mixed infections have been found in European polecats. *Trichinella* infection is still significantly present in Romania, infecting several wild omnivorous and carnivorous species in an equal manner. Regarding the spatial distribution of *T. spiralis* and *T. britovi* in Romania, both species can be found all over the country, but in wild animals, *T. britovi* was found to be more prevalent.

This research was supported by the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca (USAMV Cluj-Napoca) and by the project PPILOW. The project PPILOW has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 816172.

Keywords: Romania, *Trichinella britovi*, *Trichinella spiralis*, Wild animals

Session I: Epidemiology and Human Trichinellosis

***Trichinella britovi* – still a permanent presence in wild carnivores from the Banat area of Romania**

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Among the parasites with zoonotic risk, the *Trichinella* nematode is still diagnosed in Romania, both in domestic animals and in different species of omnivores and wild carnivores. The climate and relief of the country, especially of the Banat region, are favorable for the presence of a considerable number of wild animals that can be the host of the parasite and that represent the main actors in the sylvatic cycle of the nematode. The studies carried out in our country reveal a significant infection of wildlife with *Trichinella*, with different prevalence percentages over the years. The species *T. spiralis* and *T. britovi* are found throughout the country, but among wild animals, *T. britovi* is the most widespread.

The purpose of the study was to evaluate the presence of the *Trichinella* nematode over a period of two years and to identify the species involved in the infestation of wild carnivores from hunting funds in three counties of Romania.

A number of 141 carnivores were examined, represented by 73 foxes (*Vulpes vulpes*), 46 jackals (*Canis aureus*), 14 wild cats (*Felis silvestris*), and 8 wolves (*Canis lupus*) from Timis, Arad, and Caras Severin counties, counties neighboring Serbia and Hungary.

The muscle samples were examined by direct trichinoscopy, respectively, artificial digestion. *Trichinella* nematode larvae were identified in 14/73 foxes, 28/46 jackals, and 3/14 wild cats, respectively in 7/8 wolves. The only molecularly identified species in the infested muscles of wild carnivores was *T. britovi*. In conclusion, the study brings to light that the species *T. britovi* remains a constant in the infection of wildlife in Romania, although the involvement of the host species and the prevalence of the infection are difficult to evaluate.

Keywords: *Trichinella britovi*, wild carnivores, Romania.

Session I: Epidemiology and Human Trichinellosis

Investigation of a trichinellosis Outbreak in Apulia region, Italy, February-March 2023

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Trichinella spp. in domestic and wild reservoirs in Europe poses a risk for zoonotic disease transmission. European Centre for Disease Prevention and Control (ECDC) reported that in 2020 Italy was the European country with the second highest trichinellosis notification rate (0.13 cases/100.000). In February 2023, a *Trichinella* spp. outbreak was reported in San Marco in Lamis, Apulia region, Southern Italy (~12.500 inhabitants), part of the Gargano National Park, a wooded area rich in wildlife. We describe here the outbreak investigation main findings. Demographic and clinical data, laboratory results and risk factors were collected from symptomatic patients seeking healthcare, using a standard survey form. Serological samples were collected and tested for the presence of anti-*Trichinella* spp. IgG by western blot test. We defined a confirmed case following the ECDC case definition. From 11/2 to 20/3/2023, 12 confirmed cases were reported. Nine patients were female, mean age was 47 years (range: 8-71). The mean time interval between symptom onset and laboratory diagnosis was 31 days (range: 19-71).

All cases presented eosinophilia and myalgia, most presented specific signs including diarrhea (58%), periorbital edema (58%), subconjunctival, subungual and/or retinal hemorrhages (33%), as well as non-specific signs such as fever (58%). Five patients (42%) were hospitalized. All cases were prescribed with mebendazole which led to symptom improvement. None of them declared consuming wild game meat. All cases reported having eaten pork meat bought at a local butcher's shop. Most cases (67%) recalled consuming pork meat within one month prior to symptom onset. Three cases (25%) consumed cooked unprocessed meat, 8 (66,7%) consumed processed meat (sausages and hamburgers) and one (8,3%) dried meat. None of the pork meat was available for sampling whilst *Trichinella* spp. was not detected in any environmental samples from the butcher shop.

The cases affected by this outbreak may be higher than the reported as only symptomatic cases were tested, whilst patients with mild symptoms or asymptomatic possibly self-medicated at home. Pork meat has previously been suggested as a potential source of this trichinellosis outbreak, however to date there is no clear evidence of this association. Although previous trichinellosis outbreaks have occurred in the study area, this new outbreak suggests that local populations do not always follow the hygiene rules specified for the management of animal origin products for human consumption. Local community education on trichinellosis risks and the importance of proper handling and cooking of meat may help prevent future outbreaks.

Keywords: *Trichinella*, outbreak, zoonosis, pork meat, Europe

Session I: Epidemiology and Human Trichinellosis

A trichinellosis outbreak in Sardinia that could have gone unnoticed

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In August 2022, an outbreak of trichinellosis occurred in the Orgosolo municipality (Nuoro province, Sardinia Island, Italy). According to clinical and epidemiological data, the index case (G.G.) was admitted at the emergency department of the San Francesco Hospital of Nuoro on July 5 and then he was hospitalized for almost a month for myocarditis/pericarditis with marked hypereosinophilia. On admission in addition to myocarditis, he presented facial edema, fever, diarrhea and muscle pain. The patient reported the consumption with other symptomatic persons (S.M. and R.V.) of home-made raw sausages. The second case (S.M., who was a hunter) showed fever, headache, widespread muscle pain and marked eosinophilia in early July. At that time, the regression of the symptoms in about 10 days, led to the suspicion of parainfluenza syndrome and/or an allergic reaction. The third case (R.V., mother of S.M.), who had consumed wild boar meat brought home by her son, presented the same symptomatology of her son (fever, headache and widespread muscle pain) and eosinophilia, about 20 days after the consumption of the wild boar meat.

The almost simultaneous appearance of these three cases made the family doctor to consider that it was a trichinellosis outbreak. The medical staff alerted the veterinary services of Nuoro, but unfortunately no meat samples were traced in the patient home.

On November 18, another person (friend of G.G. and S.M) of the Orgosolo municipality, who had been suffering with severe muscle pain since July came to the Nuoro Hospital. Serum samples of these four persons tested positive for the presence of anti-*Trichinella* IgG by both ELISA and Western blot at the National Reference Laboratory for *Trichinella*, Istituto Superiore di Sanità, Rome. The recurrence of these outbreaks of trichinellosis caused by the consumption of meat from hunted wild boar (Piedmont region, 88 cases in 2019-2020; Liguria region, 36 cases in 2015; Basilicata region, 4 cases in 2014; Tuscany region, 34 cases in 2012), or from pigs illegally raised in the wild (Sardinia Island, 6 cases in 2011; 1 case in 2007; 19 cases in 2005) reveals the need to periodically provide for awareness and education campaigns for hunters and consumers. A close monitoring of *Trichinella* susceptible animals by the veterinary services is also needed.

Keywords: outbreak, trichinellosis, epidemiology, *Trichinella*, Italy

Session I: Epidemiology and Human Trichinellosis

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

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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: Epidemiology and Human Trichinellosis

Human trichinellosis in Croatia: a review of the last 17 years

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Until the turn of the 20th century, trichinellosis was a disease of public health significance in Croatia. Two human deaths were reported in 1990 and 2000 and the peak of disease was recorded in 1998 with 575 cases.

Since that time, the epidemiological situation has improved though more than 200 cases have been registered in the last 17 years. The disease was treated as a household outbreak if two or more cases were recorded after the consumption of meat products made for household consumption, or a general outbreak if meat products were purchased from a public store. In total, 26 outbreaks (from 2 to 26 cases) were registered in 25 household outbreaks and one general outbreak (3 cases). In addition, 10 sporadic cases were reported. Trichinellosis was registered in 15 of 21 counties. The highest numbers were registered in the easternmost counties of Croatia (Osijek-Baranja 77 cases; Brod-Posavina 35, and Vukovar-Srijem 32 cases), all considered endemic counties since the early 2000s.

Meat from domestic swine caused the disease in 77.4%, and meat from wild boar in 15.7% of cases. In 1.4% of cases, the source of infection was mixed meat from domestic swine and wild boar and in 5.5%, the source of the disease was unidentified. Most cases (49.3%) declared that the symptoms started after consuming smoked homemade sausages and 35.5% of cases from consuming different homemade pork products. Inadequate heat treatment of meat was responsible for 6% of cases and in 9.2% of cases, there was insufficient information. The disease was registered more frequently in males (61.2%) than in females (38.8%).

In terms of patient age, the disease was registered most frequently in 40-year-olds (20.6%), followed by 30-year-olds (18.7%), and 50-year-olds (18.2%).

In nine outbreaks, the suspected meat products were submitted to the national reference laboratory (NRL) for identification of *Trichinella* species and estimation of the level of invasion. The only identified species was *T. spiralis* and the level of infection varied from 0.2 to 167.2 L/g.

Over the past decade, the epidemiological situation has improved with fewer cases reported. This is also due to improved collaboration between medical and veterinary services.

The aim of the article was to present the epidemiological data of trichinellosis in Croatia in the last period, and compare it with the previous reports and the situation in neighbouring countries and the rest of Europe.

Keywords: human trichinellosis, Croatia, *T. spiralis*, epidemiology

Session I: Epidemiology and Human Trichinellosis

Dynamics of trichinellosis in humans in Central South Bulgaria (1990-2023)

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Introduction. The systematic control and analysis of the dynamics of trichinellosis in humans is essential for its limitation.

Aim. The aim of the study was to analyze its incidence in the Central South Bulgaria (Pazardzhik region) for the last three decades and to draw conclusions about its prevention.

Results. During the studied period, a total of 453 infected patients were registered in 14 outbreaks, sporadic cases and 15 asymptomatic cases. Sources of infection were meat from domestic pigs, wild boars and an unknown source. In all trichinosis outbreaks, no trichinoscopy was performed on the meat. According to the geographic region, several outbreaks were observed in the mountainous region of Sredna Gora (Panagyurishte town, Strelcha town). Epidemic outbreaks were also registered in mountain villages in the Rhodopes, as well as in plain villages. The last small outbreak (9 cases in 2021) was observed during the COVID-19 epidemic and lasted for one month. In the mountainous regions there were mainly natural foci (sources of infection were wild boars), and secondary synanthropic foci - in the Thracian lowlands (sources - domestic pigs). In Bulgaria, Pazardzhik region was one of the most affected by trichinellosis in the 1990s. In recent years the cases have decreased significantly there.

Conclusion. Health education conducted by the Regional Health Inspectorate contributes to the limitation of morbidity of trichinellosis. Trichinelloscopy of pork remains an important tool for the prevention of this infection.

Keywords: trichinellosis, incidence, epidemiology, prevention

Session I: Epidemiology and Human Trichinellosis

On the treatment and chemoprophylaxis of trichinellosis

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Introduction. With the synthesis of anthelmintics based on benzimidazole, significant progress was achieved in the etiological treatment of trichinellosis in humans. For the last 30 years of clinical practice, we have used available benzimidazole preparations for therapy and chemoprophylaxis of trichinellosis.

Aim. The aim of this work was to present our observations on the treatment of trichinellosis with benzimidazole derivatives - thiabendazole, mebendazole, albendazole, flubendazole. The observations were on patients treated in hospital or outpatient care, as well as asymptomatic persons infected in outbreaks. Reduction of fever, swelling, myalgia and recovery were taken into consideration to evaluate the therapeutic effect of the drugs. Eosinophilia persisted longer.

Results. Our first observations were on patients admitted in hospital and treated with thiabendazole (50 mg/kg/daily for 7 days). Regardless of its good therapeutic results, most of the patients had significant side effects. When mebendazole was released on the market, we preferred to use it (25 mg/kg, for a month). We did not observe side effects, but patient compliance was low due to the long course. In the last 15 years and until now, we have used only albendazole (15 mg/kg) for 7 days to treat mild clinical forms and for 10-14 days – for moderate and severe forms. We initiated the treatment with a smaller dose (1/3 of the total dose), which in 3 days we increased to the total dose to prevent an allergic reaction to destroyed *Trichinella* larvae. In parallel, an increase in eosinophils occurred.

Faster and more efficient resorption of albendazole ensures both migrating and encapsulated *Trichinella* larvae to be affected. Therefore, we now prefer this medication for the treatment of trichinellosis.

Since the last decades, in the case of an outbreak of trichinellosis, we have been actively searching for persons who consumed infected meat. For them, we recommend chemoprophylaxis with albendazole (15 mg/kg for 3 days) or flubendazole (200 mg a day for 3 days). In our observations the effectiveness of chemoprophylaxis was absolute.

Conclusion. Albendazole is the most preferred preparation for the treatment and chemoprophylaxis of trichinellosis. Regardless of its good effectiveness, the development of new benzimidazole derivatives with activity against *Trichinella* spp. should continue. Some data on *Trichinella* resistance to currently used drugs have already been reported.

Keywords: trichinellosis, etiological treatment, chemoprophylaxis, benzimidazole derivatives

Session I: Epidemiology and Human Trichinellosis

An outbreak of trichinosis caused by *Trichinella britovi*

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Introduction. Trichinosis is a food-borne helminth infection, caused by different species of the genus *Trichinella*. The most common source of infection is raw or undercooked pork meat.

Aim. The aim of the study was to describe a small outbreak of trichinosis caused by *Trichinella britovi*. It was registered in Sestrimo (a village in Central South Bulgaria) in the beginning of 2021, during COVID-19 pandemic.

Results. A total of 9 patients were infected after consumption of raw sausages. The first five cases of the disease were observed in the second half of January. The patients were tested for COVID-19 due to clinical sign and symptoms like fever, fatigue, lethargy, myalgia, diarrhea, etc., but results were negative. Leukocytosis as well as eosinophilia were observed in these patients, therefore they were referred for a consultation with a parasitologist. Meanwhile, three new cases have been reported with clinical picture of trichinosis. Amongst the patients there were a 4-year-old child and a pregnant woman. The diagnosis was confirmed by serological test, which was negative in one case. The patients (mild and moderate cases) underwent outpatient treatment with albendazole, antihistamines, antipyretics, analgesics. After the diagnosis of the first case of trichinosis, the home-made pork sausage consumed by all patients was examined for infection with *Trichinella* spp. The causative agent of this outbreak was identified as *Trichinella britovi*. It was further clarified that wild pork had been added to the domestic pork. The outbreak ended a month and a half after the start, all patients recovered.

Conclusion. Clinical suspicion for trichinellosis should be high in patients with history of consumption of pork, especially in the winter. This parasitic disease requires a complex diagnostic and therapeutic approach.

Keywords: helminthiasis, trichinosis, *Trichinella britovi*

Session I: Epidemiology and Human Trichinellosis

HUMAN TRICHINELLOSIS IN SERBIA FROM 2001 TO 2022

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Human trichinellosis is a mandatory reportable disease in Serbia. Surveillance on human cases is implementing through the nationwide passive surveillance system. Surveillance units are all health institutions that detect cases. District institutes for public health (n=24) perform case classification based on adopted EU case definition given in the EU Commission Decision (No. 2012/506/EU). Reported cases are summarized in weekly, monthly and annually reports. The aim of this work was to describe the epidemiological data of trichinellosis in Serbia from 2001 to 2022.

Descriptive analyze of the surveillance data has been performed. Trends of trichinellosis cumulative incidence was analyzed for country and by districts (n=25) using Joinpoint regression models, which involves fitting a series of joined straight lines on a log scale to the trends in the annual adjusted rates. During 2001--2022, a total of 2922 (cumulative inc.39.94 per 100,000 population) cases of trichinellosis were reported. More than 90% of cases are detected within clusters or community outbreaks. The average number of 133 cases has been reported (range from 0 in 2021 to 555 in 2002). Out of total registered cases during whole period 59% were among males and 41% among females. The largest number of cases are registered during the winter months. In the period from December to March is registered 77.6% of cases. While the observed annually incidence rates for all country and Belgrade district are shown significantly decreasing trend from 2004 to 2022 and from 2003 to 2022, respectively, observed incidence rates for Zaječar, Braničevo and Toplica district are shown increasing trends from 2002 to 2022, from 2020 to 2022, and also from 2020 to 2022, respectively.

Epidemiological data of trichinellosis in Serbia during the observed period are in line with the common epidemiological patterns of human trichinellosis. Despite the annual oscillations in reported cases the overall trend is decreasing which can indicate effective measures of prevention and control.

Keywords: *Trichinellosis*, human cases, Serbia

Session I: Epidemiology and Human Trichinellosis

Trichinella infection in Serbia, from 2019 to 2022

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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

4.2 Session II: Multidisciplinary, China

Isolation and identification of BALB/c mice skeletal muscle satellite cells and preliminary study on regulation in immune microenvironment in *Trichinella spiralis* infection

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Abstract

Background: Muscle satellite cells, a kind of quiescence stem cells, existed in the between cell membrane sheath and muscle fibers. As precursor cells, muscle satellite cells fuse with muscle fiber and involve in injury response. When skeletal muscle was injured by stimulation, muscle satellite cells are activated, differentiated, fused into multinucleated myotubes cell, directly participate in skeletal muscle differentiation. *Trichinella spiralis* (*T. spiralis*), an intracellular parasitic nematode, can survive and induce cysts formation in muscle cells. The process is similar to a muscle injury and repair, which makes the satellite cells and muscle cell cycle arrest in G2/M phase to become "Nurse cell" (NC) without proliferation capacity, the NC provide a suitable parasitic space for the muscle larvae of *T. spiralis*.

Results: Muscle satellite cells were isolated from infected BALB/c mice. A combination method of Percoll continuous centrifugation and differential adhesion to harvest high purity of muscle satellite cells from hindlimb skeletal muscle of infection and control group mice. The isolated muscle satellite cells were cultured in vitro and identified by Desmin immunocytochemical dyeing.

Immunological character of the cells in 4d, 21d and 35d were analyzed by Real-time PCR and FCM. FCM analysis showed that B7-H1 of muscle satellite cells was significant increased after *trichinella spiralis* infection and expression of B7-H1 was significantly reduced after 35d of cyst formation and inflammation alleviation. The Real-time PCR results evidenced that TLR3 expression of muscle satellite cells after *trichinella spiralis* infection was upregulated. Its expression was apparently upregulated in the 21d of *trichinella spiralis* infection. The three subtypes of PPARs: PPAR α , PPAR β and PPAR γ expression were also increased in different degree.

Conclusions: The systemic knowledge is deficient on satellite cells immune microenvironment during *trichinella spiralis* infection. The study analyzes the effects on immunological regulation of satellite cells from BALB/c mice during the *T. spiralis* infection. The study will provide experimental evidence in immunology to treat autoimmune disease using *T. spiralis* or product, and be possible to study the antiviral immunity mechanism by *T. spiralis* infection. It will lead to the application of *T. spiralis* or product in controlling muscle satellite cells as a biological agent and can be used in the pathogenesis study of muscle injury related to muscle satellite cell. The study also positive contribute to treatment of muscle dysfunction, diabetes and so on by *T. spiralis* or its product.

Keywords: Satellite cells; *Trichinella spiralis*; TLR3; PPARs; B7-H1

Session II: Multidisciplinary, China

Colorimetric Immunoassay Via Smartphone for *Trichinella spiralis* in Human Sera

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Abstract

Trichinella spiralis is a zoonotic parasite with a complex parasitic life cycle and exposed to animals or humans by infectious meat. However, existing point-of-care (POC) diagnosis immunodiagnosics mainly rely on the lateral flow assay (LFA), which has limited sensitivity or quantification capability. Here, we modify the traditional ELISA using crude worm extract (CWE) for detecting human serum. The entire reaction is carried out at room temperature without the need for special instruments. A low-cost and portable smartphone readout device with an application was also developed for the imaging of experimental result and data processing. The method has been validated with clinical serum samples and it showed 100% specificity in human trichinellosis detection with sensitivity of 93.33%, no cross-reaction with other parasite or virus infections. This visual immunosensor has an important application value for POC diagnosis of *Trichinella spiralis*, especially in areas lacking detection equipment.

Keywords: *Trichinella spiralis*; Colorimetric immunoassay; POC diagnosis; Smartphone

4.3 Session III: Phylogeny, Genomics and Proteomics

Identification of *Trichinella* taxa by ITS-1 amplicon next-generation sequencing with higher sensitivity for under-represented species/genotypes in mixed infections

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We applied an amplicon-based next-generation sequencing (NGS) approach to identify taxa of zoonotic nematodes of the *Trichinella* genus. The new assay entails deep sequencing of an amplified variable fragment of the ribosomal RNA operon's internal transcribed spacer 1 using the Illumina platform. The assay was evaluated using first-stage larvae (L1) of select laboratory strains of various *Trichinella* taxa mixed in known proportions and then validated using archived L1 from 109 North American wildlife hosts. The species/genotypes of these L1 isolates from wildlife had been previously determined in our laboratory using the multiplex PCR (mPCR) assay recommended by the International Commission on Trichinellosis. Two of the 109 L1 pools did not amplify in mPCR, resulting in 107 samples with both mPCR and NGS genotyping results available. For NGS, taxa were assigned using an arbitrary cut-off represented by a minimum of 0.5% of quality-filtered merged reads mapped to a reference sequence in the custom database.

NGS data analysis for the *Trichinella* laboratory strains studied revealed a sequence representation bias. *Trichinella pseudospiralis* was the most under-represented when L1 of this species were mixed with *T. spiralis*, *T. murrelli*, *T. nativa* and *Trichinella* T6 in equal quantities. However, five L1 of *T. pseudospiralis* were readily revealed by NGS in a mix with 2000 L1 of *T. nativa* (1:400 ratio).

For the L1 samples from wildlife, all *Trichinella* taxa revealed by mPCR were also identified by NGS in 103 of 107 (96.3%) samples. In 11 samples, NGS identified additional taxa, whereas additional taxa were revealed by mPCR in only 4 samples.

Although most isolates were determined to comprise single or mixed infections of *T. nativa* and/or *Trichinella* T6, *T. chanchalensis* was detected in combination with *Trichinella* T6 in a wolverine and in combination with *T. nativa* and *Trichinella* T6 in a marten (*Martes americana*) from the Northwest Territories, Canada (a new host species record for *T. chanchalensis*). In contrast, L1 from the wolverine did not amplify in mPCR, and only *T. nativa* was identified by mPCR in the marten.

A known limitation of mPCR is that it does not differentiate *T. nativa* from *T. chanchalensis*. Therefore, this new NGS assay demonstrates strong potential as a single assay for identifying all recognised *Trichinella* taxa, as well as improved sensitivity for detecting under-represented genotypes in mixed infections.

Keywords: *Trichinella*, next-generation sequencing, genotyping, wildlife surveillance

Session III: Phylogeny, Genomics and Proteomics

Gene recombination and infectious dose defines the genetic structure of *Trichinella* isolates: experimental evidences

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Trichinella spp. displays a very peculiar ecology with a gene pool partitioned over a multitude of independent demes. Each deme is enclosed in an infected host and comprises a variable number of related muscle larvae. The degree of relatedness depends on several factors like the number of passages occurred up to the present and the number of parent pairs who generated them. The higher the number of passages and the lower the number of parent pairs, the higher will be the degree of inbreeding a deme shows. Long-term isolation and high degree of inbreeding of a deme should promote the speciation. Gene flow and gene recombination oppose to speciation. In *Trichinella*, gene recombination is ensured by the occurrence of multiple infections. In recent years, studies of microsatellite polymorphisms of more than 200 *T. spiralis* and *T. britovi* cohorts from different European regions have shown very heterogeneous patterns of genetic structure, resulting probably from gene isolation and recombination events. We believe that understanding the ecological significance of this scenario allows for a better understanding of the biology of these parasites.

A controlled experiment was conducted in mice to evaluate the changes that occur in the genetic structure after passages through successive hosts. Briefly, two *T. britovi* isolates recognized by Structure analysis as belonging to separate genetic clusters were selected and used as parental strains (P1 and P2). Two mice were therefore orally infected with 200 larvae collected from P1 and P2 (100 larvae each) to generate the F1 in two separate lineages (A and B). Forty-five dpi, muscle larvae were recovered separately from each mouse and 200 of them used to generate the F2. Generations from F2 to F9 received a decreasing number of infecting larvae, from 200 (F2) to 6 (F9). Any modification of the genetic structure at each generation were evaluated by Structure analysis.

Interestingly, both F1s showed an admixed pattern where all larvae were assigned to alternative clusters, while generations F2 to F8 showed a homogeneous genetic structure in which no clustering is detectable. Finally, F9s, derived from very low infectious dose, showed a loss of genetic variability in both lineages but even if the genetic structure of the single lineage is homogeneous the two lineages appeared genetically divergent.

Present results suggest that the genetic structure pattern displayed by cohorts remain stable until recombination or a very low infectious dose occur when producing a new generation.

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

Survival of *T. spiralis* and *T. pseudospiralis* in experimentally infected wild boar muscle tissue under freezing and environmental conditions

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Wild boars have been found to be one of the links for transmission of *Trichinella* spp. between humans and wildlife. According to several authors *T. spiralis*, *T. britovi*, *T. nelsoni* and *T. pseudospiralis* are the main species affecting wild boars. The purpose of this study was to determine whether *T. spiralis* and *T. pseudospiralis* can survive in decaying wild boar tissue and to assess the freezing tolerance of these *Trichinella* species in experimentally infected animals. Two wild boars were used, one was infected with 20,000 muscle larvae (ML) of *T. spiralis*, and the other one with 20,000 ML of *T. pseudospiralis*. Both were euthanized 19 weeks post infection (p.i.). One of the forelimbs and one of the hindlimbs of each wild boar were severed and placed over a soil layer in plastic containers to assess the ML's ability to survive in decaying tissue. All containers were covered with metallic mosquito net screens and placed in a room protected from rain and sunlight. These were exposed to natural environmental temperature and humidity conditions. Every week samples from both limbs were taken and artificially digested for the recovery of larvae. The recovered ML were then inoculated into 3 mice to determine their reproductive capacity index (RCI).

To study the freezing tolerance of the ML of both *Trichinella* species, the upper forelimb and the upper hindlimb were used. From each of these muscle groups, 6 2-cm thick, 200 g samples were taken and placed in individual plastic bags and stored at -18°C for 14 days. A sample of each muscle was analyzed by means of artificial digestion after 2, 4, 7, 9, 11, 14 days respectively. The recovered larvae were inoculated into 3 mice to evaluate their RCI. Results showed that *T. spiralis* remained infective in decaying wild boar tissue for 11 weeks, while *T. pseudospiralis* remained infective for 4 weeks. The freezing tolerance assay revealed that *T. spiralis* larvae remained infective for 9 days at -18°C, while *T. pseudospiralis* larvae lost their infectivity after just 2 days at the same temperature. Consequently, their capacity to survive in decaying and frozen tissues would allow for this parasite to remain active in nature, and potentially be spread by scavenging animals.

Keywords: *Trichinella*, freezing tolerance, persistence study, infectivity, wild boars

The present research was approved by the Committee for the Use and Care of Laboratory Animals (CICUAL) of the Facultad de Ciencias Veterinarias, University of Buenos Aires, under permit number 2015/16.

This work was supported by Universidad de Buenos Aires, Secretaría de Ciencia y Técnica Subsidio UBACyT 200 201902 00396 BA and UBACyT 20020170100530BA and Ministerio de Ciencia y Tecnología, FONCyT Subsidio PICT-2018- 01203

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

Comparison of the adult stage distribution pattern of three *Trichinella* species during the intestinal phase in CF1 mice

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Trichinella spp. is a parasite that triggers immunological processes that vary depending on the host involved, the parasite species, and the infective dose. It is believed that the development of the immune response at the intestinal level is correlated with the burden of adult stage worms. The objective of the present study was to compare the adult worm burden in different sections of the intestine over time caused by *T. spiralis*, *T. pseudospiralis*, and *T. patagoniensis* in CF1 mice. For that, 3 groups of 16 animals were orally inoculated with 300 L1 larvae of one of the 3 species under study, and 4 animals were given saline physiological solution (control group). Euthanasia was performed on 4 infected animals and on 1 from the control group on days 3, 6, 9, and 12 post-infection (pi). Adults were obtained from the different sections of the intestine (duodenum, jejunum, ileum, cecum, and colon) according to Fariña et al., 2020. The median count of adult worms in the intestine was higher for animals infected with *T. spiralis*, followed by *T. patagoniensis* and *T. pseudospiralis*. The maximum value of the median total adult count was registered in the jejunum, on day 3 pi for *T. spiralis* (108) and on day 6 pi for *T. patagoniensis* (91) and *T. pseudospiralis* (68). It was observed for all three *Trichinella* species that the median of males exceeded the median of females only in the ileum.

For animals infected with *T. spiralis* and *T. patagoniensis*, the median number of adults per day per organ decreased over time in the duodenum and jejunum, but increased in the ileum, cecum, and colon.

The results of this study showed differences and similarities with other studies using different host species and *Trichinella* species and different infection doses. However, none of these studies details adult worm burden by intestine section. The adult worm kinetics could indicate a pattern of colonization and anterograde displacement over time that may be influenced by simultaneous immune response processes involving different parts of the intestine during the intestinal phase. Further characterization of other aspects of this phase could contribute to the knowledge of the immune mechanisms involved.

Keywords: *Trichinella* adults - intestinal phase - adult recovery

This research has the approval of the Institutional Committee for Care and Use of Laboratory Animals (CICUAL) of the FCV-UBA, under protocol 2021/31. This work was supported by Universidad de Buenos Aires, Secretaría de Ciencia y Técnica Subsidio UBACyT 20020190200396 BA and UBACyT 20020170100530BA and Ministerio de Ciencia y Tecnología, FONCyT Subsidio PICT-2018- 01203

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

Comparative analysis of early diagnosis of invasion by species *Trichinella nativa* and *Trichinella spiralis* by serological and biochemical parameters

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Early diagnosis of trichinellosis is based on the use of serological methods based on the detection of antibodies in the blood serum of patients with signs of trichinella invasion, with a history of eating raw or undercooked meat from wild animals and pigs. The use of the ELISA method makes it possible to obtain a reliable result on average 45 or more days after the invasion. With earlier diagnosis, the results can be both false positive and false negative, and much depends on the technical equipment of the laboratory and the qualifications of the staff. The studies were carried out on 3 groups of experimentally infected rabbits (3 heads in each group), one group was a control, for the experimental groups a dose of 2500-3000 *Trichinella nativa* and *Trichinella spiralis* larvae, respectively, was used. Experiments carried out using the blood of experimentally infected rabbits with *Trichinella nativa* and *Trichinella spiralis*, on days 7, 14, 31, 45 and 70 after the invasion, made it possible to identify the main parameters that also make it possible to determine the species of Trichinella.

Biochemical parameters of the blood of rabbits infested with *Trichinella nativa* and *Trichinella spiralis* isolates were taken from 7 to 70 days. In the case of *Trichinella nativa* invasion, significant differences were found in the level of creatine kinase CK-NAC, which increased from 18.2% to 191%.

With the invasion of *Trichinella spiralis*, a decrease in this indicator from 0.8 to 5.4% relative to the control was noted. However, with *Trichinella spiralis* invasion, the reason for early diagnosis may be an increase in the level of neutrophils on day 7 by 26.7% and on day 31 by 39.4% and lymphocytes by day 7 by 20%. The decrease in the level of platelets in the blood in both cases of infection from 7-70 days was noted at the level of 12.4-21.6%.

Perhaps this is due to the secretion of parasite larvae into the environment, which reduce the viscosity of the blood, to move through the bloodstream and invade the muscles. Both cases of invasion are also characterized by a decrease in the total number of formed cells in the blood (hematocrit).

At the same time, a comparison of the results of blood with ELISA using the ES antigen for diagnosing trichinellosis showed the presence of antibodies in the blood serum of infected animals already on the 7th day after the invasion. However, in the group of rabbits infected with *T. nativa*, the immune response was significantly lower than with *T. spiralis* infection, respectively 1:320 and 1:650. It is not possible to make an unambiguous conclusion about the presence of the disease with such a titer, because there is a possibility of false positive or false negative results. Reliable results were obtained only on the 45th day with an increase in titer up to 1:12800 with the invasion of *Trichinella nativa* and 1:25600 with the invasion of *Trichinella spiralis*. At the same time, the titer by the 70th day decreased by half in the case of *Trichinella spiralis*, and remained unchanged after infection with *Trichinella nativa*.

As a result of the comparative analysis of the serological method and biochemical blood parameters, it can be concluded that with early diagnosis on days 7-31, it is possible to use biochemical blood tests for such indicators as CK-NAC creatine kinase in case of infection with *Trichinella nativa* and in the case of *Trichinella spiralis*, an increase in the level neutrophils and lymphocytes (on days 7-14). In both cases, it is important to note the level of platelets, which in both cases decreases starting from the 7th day after the invasion. Thus, the results of the study showed the possibility of using a blood test to confirm invasion along with the ELISA method.

Keywords: trichinellosis, *Trichinella nativa*, *Trichinella spiralis*, ELISA, blood parameters.

The study was funded by the Ministry of Education and Science of the Republic of Kazakhstan to frame the project of the Young Scientists No. AP09058176 "Express test for the diagnosis of trichinellosis" for 2021-2023 and project of grant funding AP14870972 "Development of enzyme immunoassay based on the recombinant antigen of *Trichinella spp.*"

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

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

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Extracellular 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.

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

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

Enrichment and characterization of muscle extracellular vesicles during *Trichinella spiralis* infection in mice

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During the muscle invasion by *Trichinella spiralis* newborn larvae (NBL), the transformation of muscle cells to nurse cells is still not understood, in particular the molecular interplays between host and parasite remains not identified. The emerging role of extracellular vesicles (EVs) in the latest decades opens a new field in the understanding of how the pathogen makes itself a niche indispensable for its survival. Produced by almost all cell types, it has been identified as a new way of cell-cell communication in mammals and a new way of host-pathogen interactions in helminths.

Muscle cells themselves produce EVs during physiological mechanisms, like regeneration (to which infection with *Trichinella* has been compared), or pathological conditions, like denervation, inflammation or insulin resistance. In regeneration, muscle cells exchange EVs with satellite cells to induce their proliferation or with immune cells to induce an inflammatory response.

Moreover, muscle EVs are enriched in vimentin, an intermediate filament protein. We have previously shown that a NBL stage specific protein (NBL-1) induce the expression of vimentin in muscle cells *in vitro*.

We therefore investigated muscle EVs in OF1 mice during oral infection by *Trichinella spiralis* (ISS004). EVs were enriched by ultracentrifugation after muscle digestion and analysed for their size and number by tunable resistive pulse sensing (tRPS), purity through the absence of cell-associated proteins (immunodetection in Western Blot), EVs-associated markers (CD9, CD63, Tsg101) and the vimentin protein content. The presence of *Trichinella* antigens has also been tested.

Muscle EVs properties vary during the infection cycle. Vimentin and CD9 protein content of EVs (normalised to EVs protein rate) varies. CD9 tetraspanin is also known as a regulator of cell fusion during muscle regeneration.

These results will allow to initiate a more global analysis of local EVs in muscle by proteomic and transcriptomic to understand how the parasite and the cell populations interact through this way. New biomarker or vaccine target candidates may thus be identified.

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

***Trichinella spiralis* muscle larva-derived deoxyribonuclease II promoted differentiation of C2C12 myoblasts**

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After invasion of *Trichinella spiralis* newborn larva, the infected muscle cell is induced to develop into nurse cell which is prepared for its long-term survival. Nurse cells are morphologically and functionally quite distinct from muscle cells. Our previous studies have shown that the process of transformation of the infected muscle cells into nurse cells involves in activation, differentiation and proliferation of muscle stem cells, and dedifferentiation, redifferentiation, apoptosis, and transformation of the infected muscle cells. It is considered that excretory-secretory (ES) products of muscle larvae are responsible to the process. However, the detailed molecular biological mechanism of nurse cell formation and what kinds of molecules are involved in remain to be elucidated. Some studies suggest that the deoxyribonuclease II (DNase II), a 43 kDa ES protein is likely to play important role in such muscle cell transformation. In this study, we investigated the effects of the DNase II on myocyte differentiation, apoptosis, and cell transformation. Proteomic analysis of ES products showed that adult worm and muscle larvae secrete a 43 kDa protein with DNase II domain. Expression kinetics indicated that there are developmental stage-specific of the DNase II secreted by adult worm, newborn larva and muscle larva. Transfection of a muscle larva-specific DNase II expression plasmid into a myoblast cell line (C2C12) resulted in the promoted expression of muscle differentiation-related genes (myogenin, MyoD, Mef2c, Myh2, Myh3), while the transfection had no effects on apoptosis (Tnfa, Cas3, Cas9, Bcl2, BAX, Apaf -1), cell cycle and transformation-related genes (p21, p27, cyclin D, cyclin E, c-Ski, Tgfb, Smad), suggesting that the DNase II is likely involved in the redifferentiation during nurse cell formation.

Future analysis of specific DNase II to the developmental stages of *Trichinella* may elucidate their roles in their different parasitism.

Keyword: *Trichinella spiralis*, ES products, deoxyribonuclease II, muscle differentiation

4.5 Session V: Detection, Legislation and Control

Assessment of proficiency testing samples for digestion assay using freeze-tolerant sylvatic *Trichinella* spp. with low infectivity for domestic swine

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Use of proficiency testing (PT) samples containing *Trichinella spiralis* (the most infective species for pigs and a reportable parasite in Canada and elsewhere) is the current standard for assessing performance of the pepsin/HCl digestion assay to recover live first-stage larvae (L1) from infected muscle. However, since L1 of all *Trichinella* taxa are morphologically indistinguishable, using a freeze-tolerant alternative to *T. spiralis* that has low infectivity for pigs, such as *T. nativa*, would reduce the biorisk to the national swine herd and could potentially enable preparation of large batches of samples that could be stored frozen yet yield the live larvae required for PT. Based on our well-established, validated and robust PT procedure using encapsulated *T. spiralis* L1, we prepared samples using both *T. spiralis* L1 and *T. nativa* L1 propagated in rats and mice, respectively. Five samples per species, each consisting of 20 g of ground meat containing 10 L1, were tested after refrigeration (5 ± 3 °C) for 24 hr and after refrigeration or freezing (-20 ± 10 °C) for 25 weeks. The refrigerated *T. spiralis* and *T. nativa* samples tested shortly after preparation yielded average L1 recoveries of 94 % and 72 %, respectively, with 100 % motility. The *T. spiralis* and *T. nativa* samples refrigerated for 25 weeks yielded average L1 recoveries of 60 % and 80 %, with 39 % and 64 % motility, respectively. The *T. spiralis* and *T. nativa* samples frozen for 25 weeks yielded similar average L1 recoveries of 68 % and 82 %, respectively; however, no motility was observed for either species.

Although the freshly prepared *T. spiralis* PT samples yielded higher larval recoveries than the *T. nativa* samples, the latter yielded higher larval recoveries after 25 weeks of either refrigeration or freezing. However, none of the *T. nativa* samples stored at -20 °C yielded live larvae.

This unfortunately corroborated previous findings at our laboratory whereby freeze-tolerant taxa lost this characteristic when propagated in rodent models. Nonetheless, the use of *T. nativa* as an alternative to *T. spiralis* for PT sample production continues to show promise based on the higher numbers of motile larvae recovered after long-term refrigeration, although further work is required to assess larger sample sizes and additional time points.

Keywords: *Trichinella spiralis*, *Trichinella nativa*, proficiency testing, digestion assay

Session V: Detection, Legislation and Control

Early detection of *Trichinella* infection through real-time PCR analysis in experimentally infected pigs and wild boars

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Early detection of trichinellosis is of utmost importance, emphasizing the significance of its diagnosis. Pigs and wild boars are the most commonly infected animals carrying the parasite. In Argentina, *T. spiralis*, *T. pseudospiralis*, *T. patagoniensis*, and *T. britovi* are the species that have been isolated. The objective of this study was to implement a real-time PCR assay that could detect new born larvae circulating in experimentally infected pigs and wild boars. Twenty thousand larvae of a *Trichinella* species were inoculated in thirteen wild boars and six pigs. *T. spiralis*, *T. pseudospiralis*, and *T. patagoniensis* were inoculated in four, five, and five wild boars, respectively, with one wild boar used as a negative control. Among the pigs, two were inoculated with *T. spiralis*, two with *T. pseudospiralis*, and one with *T. patagoniensis*, with one pig used as a control. Blood was drawn from the jugular vein of all wild boars on days 7 and 14, and on days 5, 9, 12, and 15 for pigs. These samples were then centrifuged, and the serum was obtained and kept frozen until used. The DNeasy® Blood and Tissue Kit (QIAGEN, Inc., Valencia, CA, USA) was used to extract DNA following the manufacturer's instructions. The 18S primer was utilized to detect *T. pseudospiralis*, *T. patagoniensis*, and *T. spiralis* DNA, and the Rep primer was used to detect *T. spiralis*. Each serum was then analyzed using RT-PCR. The results varied depending on the *Trichinella* species and animal involved.

Circulating DNA could be detected with Rep during days 7 post-infection from all wild boars infected with *T. spiralis* and from just one wild boar with 18S, while for *T. pseudospiralis* and *T. patagoniensis* DNA could not be detected in any wild boar. No circulating DNA was detected in the serum of pigs that were experimentally infected. Since the results are still variable, we are evaluating the use of new primers to increase the detection of newly born larvae, more studies are still necessary.

Keywords: *Trichinella*, LRN detection, wild boars, Rep

This research has the approval of the Institutional Committee for Care and Use of Laboratory Animals (CICUAL) of the FCV-UBA, under protocols 2018/22 and 2015/16. This work was supported by Universidad de Buenos Aires, Secretaría de Ciencia y Técnica Subsidio UBACyT 20020190200396 BA and UBACyT 20020170100530BA and Ministerio de Ciencia y Tecnología, FONCyT Subsidio PICT-2018- 01203

Session V: Detection, Legislation and Control

Cutaneous abdominal biopsy enabled the diagnosis of clinical *Trichinella britovi* infection in a hunting dog

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A six-year-old female mixed-breed hunting dog, which has been imported from Bulgaria into Germany, was admitted to a private veterinary clinic presenting a swelling (approx. 10 x 10 x 1 cm) with ulceration of the skin on the left abdominal flank. The dog evidenced difficulty to climb the stairs. The mass including skin and subcutaneous tissues was surgically removed, and submitted for routine histological examination. Microscopically, a high-grade pyogranulomatous to mixed-cellular and lymphoplasmacytic cell infiltration, with fibrosis and hyalinization involving cutis, subcutis and cutaneous muscle was observed. Multiple cyst-like structures (~0.25 mm diameter) with an eosinophilic hyalinised wall, containing nematode larvae sections (~26 µm wide) were detected intralesionally, and a *Trichinella* infection was suspected. Consequently, DNA was extracted from formalin-fixed paraffin-embedded tissues and a multiplex PCR for *Trichinella* species/genotype identification was performed. After electrophoresis, two bands of 129 and 253 bp were observed, which corresponds to the banding pattern of *T. britovi*. *Trichinella* T8 and T9 show a similar banding pattern; however, these genotypes are assumed to exclusively circulate in sub-Saharan Africa and Japan, respectively, and there are no reports from Europe so far. The localisation of *Trichinella* in the cutaneous muscle, and the high degree and extension of inflammatory reaction observed are unusual. Consumption of hunted prey was assumed as the most probable source of infection. *Trichinella* spp. should be considered in the differential diagnostic of nematodes, which may be observed in cutaneous and muscular biopsies in dogs.

Session V: Detection, Legislation and Control

Activities of the European Union Reference Laboratory for Parasites (EURLP) on *Trichinella* during 2019-2022

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Key words: *Trichinella*, European Union Reference Laboratory for Parasites, National Reference Laboratories, proficiency testing, diagnostic methods.

Since its designation in 2006, the EURL-NRL network for Parasites represents an excellence and a repository of knowledge and expertise. The EURLP quality system, compliant with the EN ISO/IEC 17043 and 17025, has been developed to ensure support to the National Reference Laboratories (NRLs) of the EU member states (MSs) in the field of foodborne parasites. Regarding *Trichinella*, a total of 2,548 samples were tested during 2019-2022 by one or more of the accredited tests and by ISO 18743:2005. Nevertheless in 2020 and 2021, scientific and technical training activities were affected by the COVID-19 pandemic, which lead to restrictions of movements across Europe and partial or total lockdowns, the EURLP produced and supplied to MSs reference materials as infected mouse carcass (about 60); positive swine sera (about 60); crude and excretory/secretory antigens (about 10 mg); DNA and muscle larvae of all *Trichinella* species and genotypes. The EURLP activity as PT provider is of considerable importance, as the participating NRLs are in turn in charge of verifying the performance of the appointed laboratories for official food and feed controls. In the referred period, the EURLP organized, on yearly basis, PTs in compliance with the ISO 17043. About 420 laboratories were involved in the PT on the detection of *Trichinella* larvae in meat according to EU Regulation 2015/1375, and 90 laboratories participated in the PT on the identification of *Trichinella* larvae at the species level. The EURLP provided scientific and technical support to NRLs personnel through the organization of 18 trainings on accredited diagnostic methods.

Annually, a dedicated workshop allows an exchange of information among the EURL-NRL network on the epidemiology and diagnosis of foodborne parasites circulating or at risk to be imported in the EU. Since 2006, the EURLP has developed, maintained, and periodically updated a website (<https://eurlp.iss.it/>) for sharing information within the network. Furthermore, the EURLP supports European and international organizations such as EFSA, ECDC and WOAH in the field of foodborne parasites, and collaborates with laboratories in third countries.

Session V: Detection, Legislation and Control

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

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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* priocek 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* Priocek 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 encapsulated) were tested independently in two Serbian laboratories (Faculty for veterinary medicine, Belgrade; and in National reference laboratory for *Trichinella*). The results revealed that *Trichinella* Priocek 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 than the *Trichinella* Priocek AAD kit.

But regardless, the larvae recovery obtained by the *Trichinella* Priocek 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* Priocek AAD kit

Session V: Detection, Legislation and Control

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

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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 Nađ. 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 significant impact on national regulatory decision makers to establish the Serbian Commission on Trichinellosis, 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 Srboľjub 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 Trichinellosis 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, Legislation and Control

Proficiency tests for detection of *Trichinella spiralis* according to Regulation (EU) 2015/1375 in Germany – challenges, organization and results

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Keywords: proficiency test, *Trichinella spiralis*, artificial digestion

According to Regulation (EU) 2015/1375 and Regulation (EU) 2020/1478 official *Trichinella* inspection is mandatory for all *Trichinella*-susceptible species whose meat is intended for human consumption. Methods that may be used for detection of larvae are specified within these regulations. Participation in proficiency tests (PT) provides information on diagnostic performance of laboratories. According to Regulation (EU) 2017/625, there is no accreditation requirement for official laboratories if they regularly and successfully participate in proficiency tests (PT) organized by the national reference laboratories (NRL)

Since 2020, the German NRL for *Trichinella* organizes participation in PT for currently 618 official *Trichinella* testing laboratories, with each laboratory taking part every two years. In addition to lifting the accreditation requirement, this has also the advantage that there is a Germany-wide overview of the performance of each laboratory.

When organizing PT both, the existing organizational structures of the individual federal states and the cooperation with official *Trichinella* testing laboratories need to be considered. For this purpose, coordinators were appointed by the federal states to act as focal point for the NRL and for each participating laboratory within the respective federal state.

The NRL makes arrangements with these coordinators regarding PT participation, offers regular PT appointments, sends samples to the laboratories, evaluates the results and finally sends all certification to these coordinators, which forward them to the respective laboratories.

The challenge was to develop and validate a method for providing PT samples in such a way that the high demand for PT samples was met and that such samples still reflect the characteristics of a natural” sample. At the same time samples need to continue to be comparable and with high quality. For this purpose, the NRL *Trichinella* has established a method in which pre-determined number of larvae are first pipetted into gel bodies, which are then placed in meatballs made from minced pork. These samples must later be analyzed using artificial digestion.

Since 2020, the NRL has facilitated 1260 PT, providing representative and valuable insights into the performance of *Trichinella* testing laboratories. Criteria for assessing performance include qualitative results, larval recovery rate, amount of residue after digestion on the sieve, and quantitative results. Participation in a PT was successful if all samples were determined qualitatively correct. In PT carried out to date, 95% of positive samples were identified correctly in terms of quality. This rate can be considered as a satisfactory performance for *Trichinella* testing laboratories in Germany.

Session V: Detection, Legislation and Control

Approach of early diagnosis of *Trichinella* infection by detecting microRNAs of extracellular vesicles

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Clinical diagnosis of trichinellosis, especially early diagnosis, is difficult because the disease is usually nonspecific of symptoms or asymptomatic. Currently, the diagnosis is mainly achieved by using muscle biopsy and immunodiagnostic assay to detect specific antibody. However, the low sensitivity of biopsy and the relatively long period of time between the onset of symptoms and a positive serological test limit the use of these methods for early diagnosis. After infection, muscle larvae develop into intestinal infective larva and adult worm within several days under intestinal epithelium. Here we proposed, during quite early time of infection, *Trichinella* adult worms and muscle larvae may secrete microRNA (miRNA)-containing extracellular vesicles (EV) that are released into blood stream. Therefore, miRNA can be a potential target of early diagnosis of Trichinellosis. Firstly, we collected adult worm and muscle larva EVs and sequenced the miRNA. Totally 132 and 98 kinds of miRNAs in adult worm-EV and muscle larva-EV were found respectively. Fifty-four kinds of miRNAs were shared in both EVs. Seventy-eight and 43 kinds of miRNAs were found in adult worm-EV and muscle larva-EV, respectively. Then, we selected 24 kinds of miRNA based on their high copy in EV and stage specificity for further study. By using quantitative PCR, several kinds of miRNA were detected from the muscle and intestine or serum of *T. spiralis*-infected mice at one week post infection. Our study indicated that the miRNA of *Trichinella*-secreted EV could be promising targets for early diagnosis of trichinellosis.

Keyword: *Trichinella*, extracellular vesicle, microRNA, diagnosis

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CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

616.995.132(048)
636.09:616.995.132(048)

INTERNATIONAL Conference on Trichinellosis (16 ; 2023 ; Beograd)

Program and Abstract Book / 16th International Conference on Trichinellosis, ICT-16, August 30th - September 1st, 2023, Belgrade, Serbia ; Organized by University of Belgrade, Institute for the Application of Nuclear Energy [i. e.] INEP, National Reference Laboratory for Trichinellosis. - Belgrade : University, Institute for the Application of Nuclear Energy - INEP, 2023 ([Beograd] : Unagraf). - 152 str. : ilustr. ; 21 cm

Tiraž 80. - Registar.

ISBN 978-86-918341-1-1

а) Трихинелоза -- Апстракти

COBISS.SR-ID 123197193