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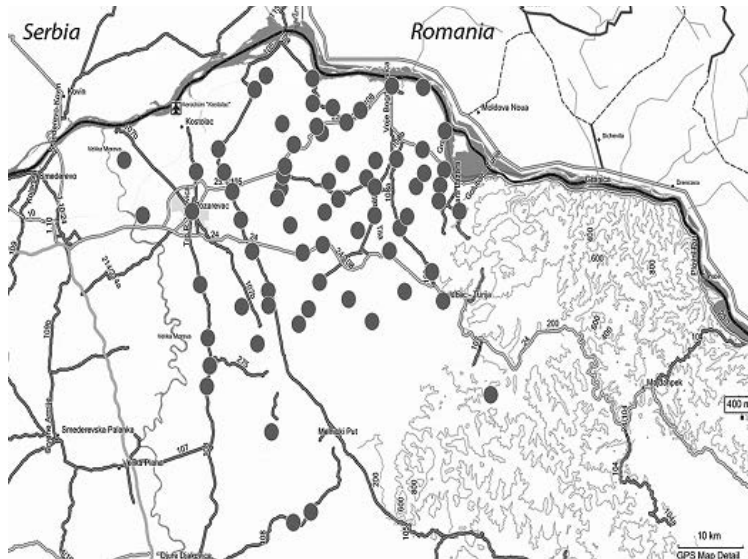
Immune response to *Trichinella* and Sars-CoV-2 antigens in patients involved in the trichinellosis outbreak in Serbia, March 2022

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DISTRICT BRANICEVO – A RISK SPOT FOR TRICHINELLOSIS IN SERBIA

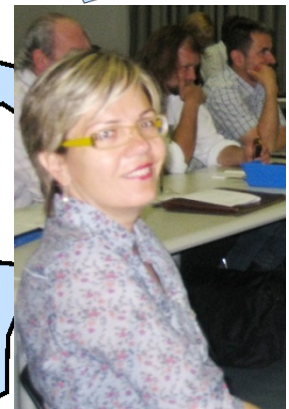
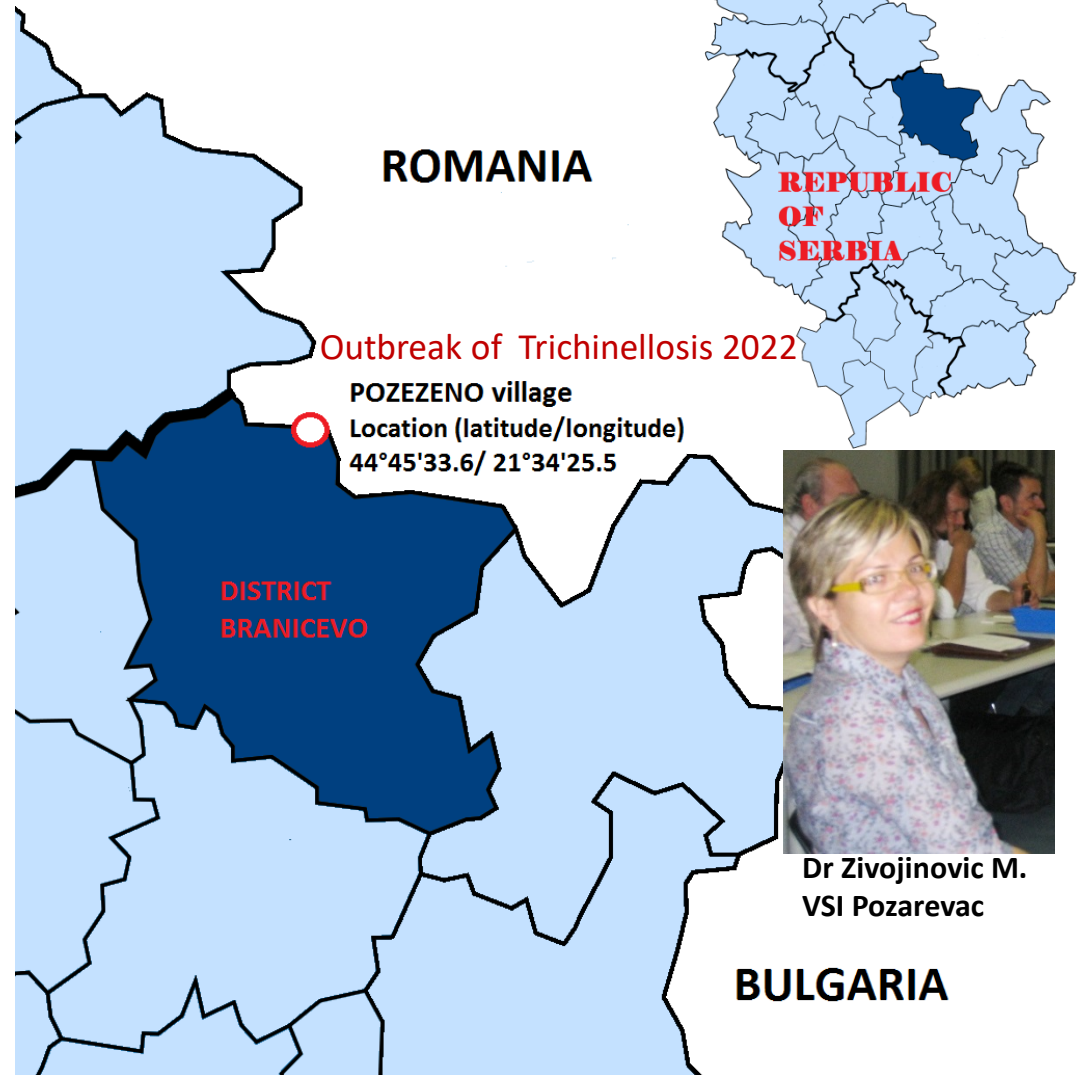
District Branicevo belongs to the region (Eastern Serbia), with the fluctuating rate of *Trichinella* infections in swine.



Zivojinovic M., et al. (2010)., *Parasite* 17:369.
Settlements in Branicevo (black spots), where the presence of *Trichinella* infected swine was recorded.

Both *T. spiralis* and *T. britovi* were identified in wild life (including wild boars).

Zivojinovic M., et al. (2013). *Vet. Par.* 159(3-4):358-60



Dr Zivojinovic M.
VSI Pozarevac

HISTORICAL OVERVIEW OF TRICHINELLA AND TRICHINELLOSIS IN DISTRICT BRANICEVO IN THIS MILLENNIUM

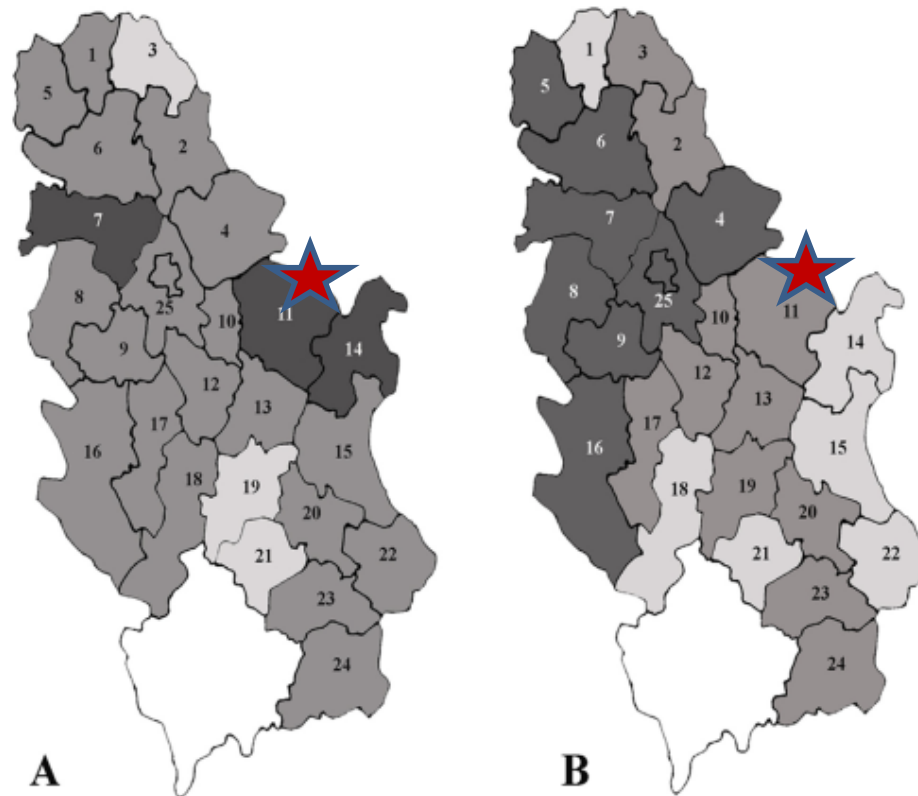
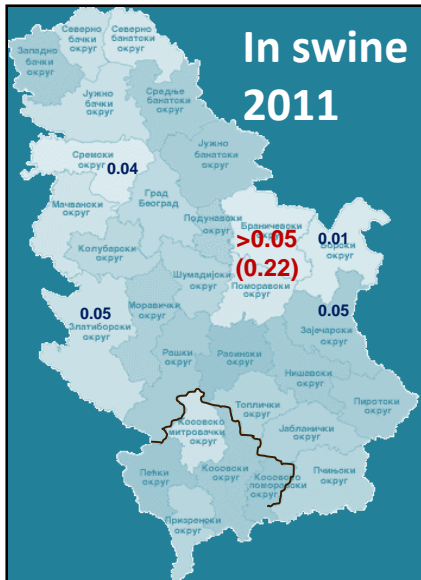


Fig. 1. Distribution of *Trichinella* infection in 25 districts of Serbia during the period 2006–2010. (A) *Trichinella* infection in swine – distribution and prevalence (dark gray fields – prevalence > 0.05, medium gray fields – prevalence < 0.05, light gray fields – infection was not detected, white field – Kosovo, data not available); (B) *Trichinella* infection in humans – geographical distribution according to the frequency of outbreaks (dark gray fields – infection detected for 3 years or more, medium gray fields – infection detected 1 or 2 years, light gray fields – infection was not detected during five year period, white field – Kosovo, data not available).

Lj. Sofronic-Milosavljevic et al. / *Veterinary Parasitology* 194 (2013) 145–149

Prevalence of *Trichinella* infection - District Branicevo

During the period 2011-2015



• HUMAN TRICHINELLOSIS

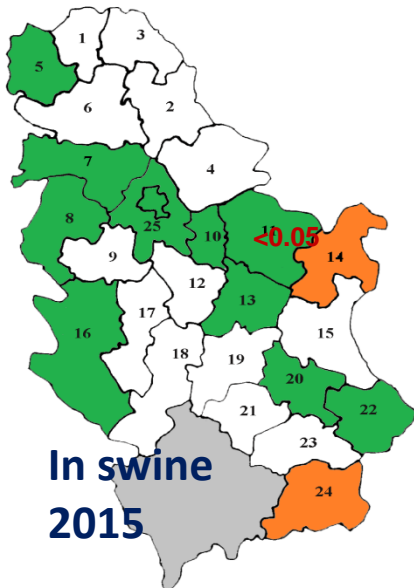
In Serbia

There was 41 outbreaks (450 cases)

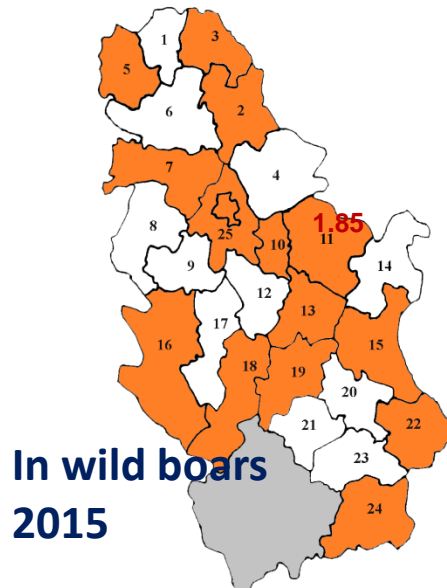
In the District Branicevo

2 outbreaks

In 2011 (10 cases)



In swine
2015



In wild boars
2015

In 2015 (11 cases)

HUMAN TRICHINELLOSIS

- **During the period 2016-2020**

In Serbia there was 13 outbreaks (264 cases)

None were registered in the District Branicevo

- **In 2021, not a single case of trichinellosis was registered in the entire territory of Serbia**
- **Still**, despite the good implementation of preventive measures by the veterinary services **in this District, the risk of an outbreak appearance** - due to consumption of uninspected, raw or undercooked meat or meat products from prepared from domestic or wild boars - **is not negligible**, as can be seen from the following data:

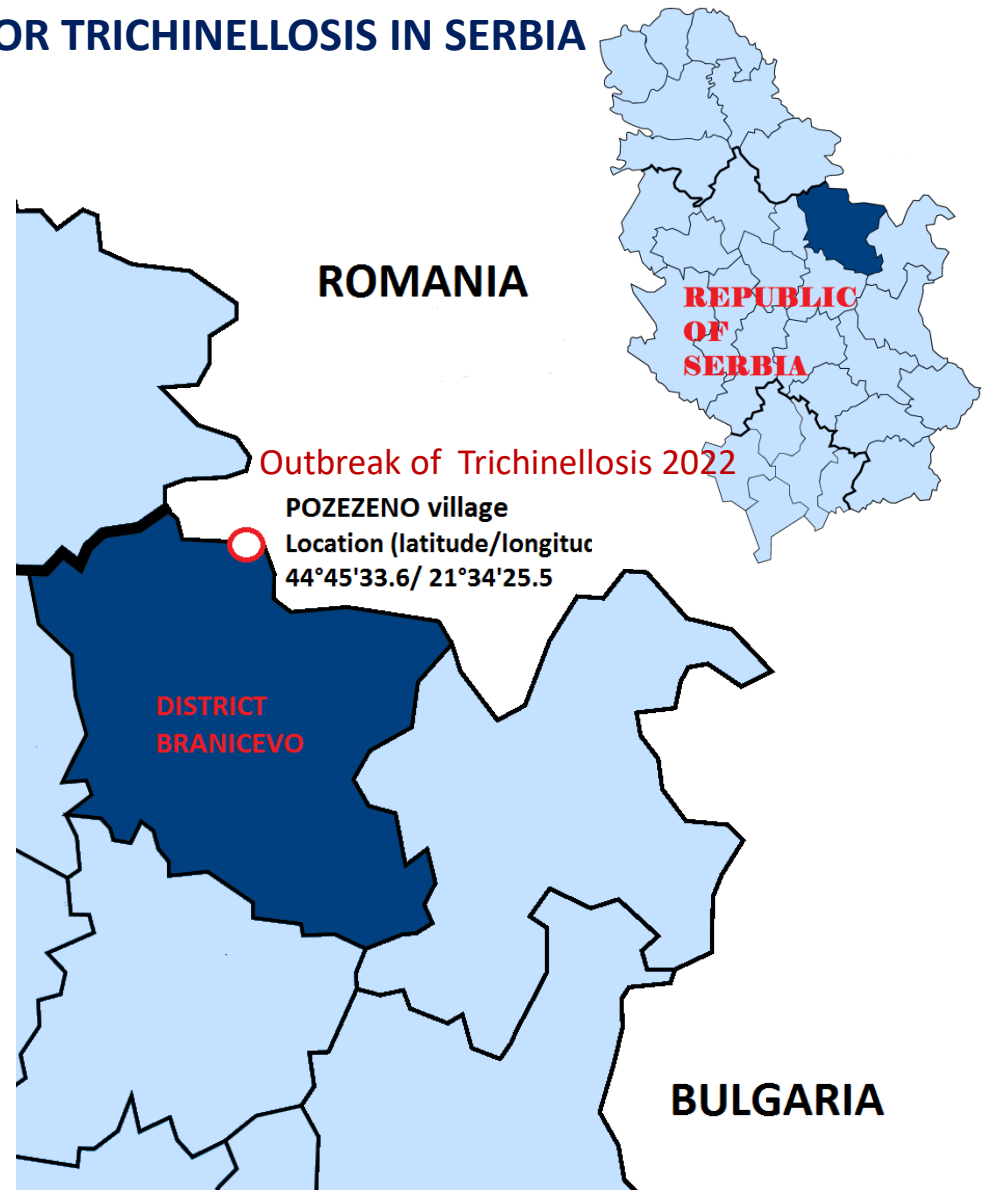
DISTRICT BRANICEVO – A RISK SPOT FOR TRICHINELLOSIS IN SERBIA

Data indicating the risk of infection with *Trichinella spp.* in humans in the period from 2016.

App. 120-150,000 DOMESTIC swine are slaughtered annually in Branicevo. More than half are slaughtered in slaughterhouses (**raised in controlled conditions**) and the prevalence of infection is between 0.0 and 0.001% - **no risk of human infection.**

About 35,000 pigs from small farms (**back yard raised**) are sacrificed annually, and the prevalence of *Trichinella* infection **varies from 0.027% to 0.1%**. - there is a **risk of human infection** if uninspected meat is consumed.

Every year, on average, about 450 **wild boars** are caught, and the prevalence of *Trichinella* infection in them **varies from 0.7% to almost 8.0%**.



Outbreak of Trichinellosis in Pozezeno, March 2022

Trace back study performed by Veterinary Specialist Institute, Pozarevac

The source of human infection in this outbreak was traditional meat product “Kulen”.



Traditional Kulen preparation procedure. It is a kind of the sausage prepared from well minced pork that should stay in the brine for 7 days (becomes impregnated with salt), be hanged in draft to dry, smoked for 1 month and exposed to process of “maturation” for at least 3 months (in a well air ventilated and cool area at app. 4-8 °C i.e. usually during the winter time).

Outbreak of Trichinellosis in Pozezeno, March 2022

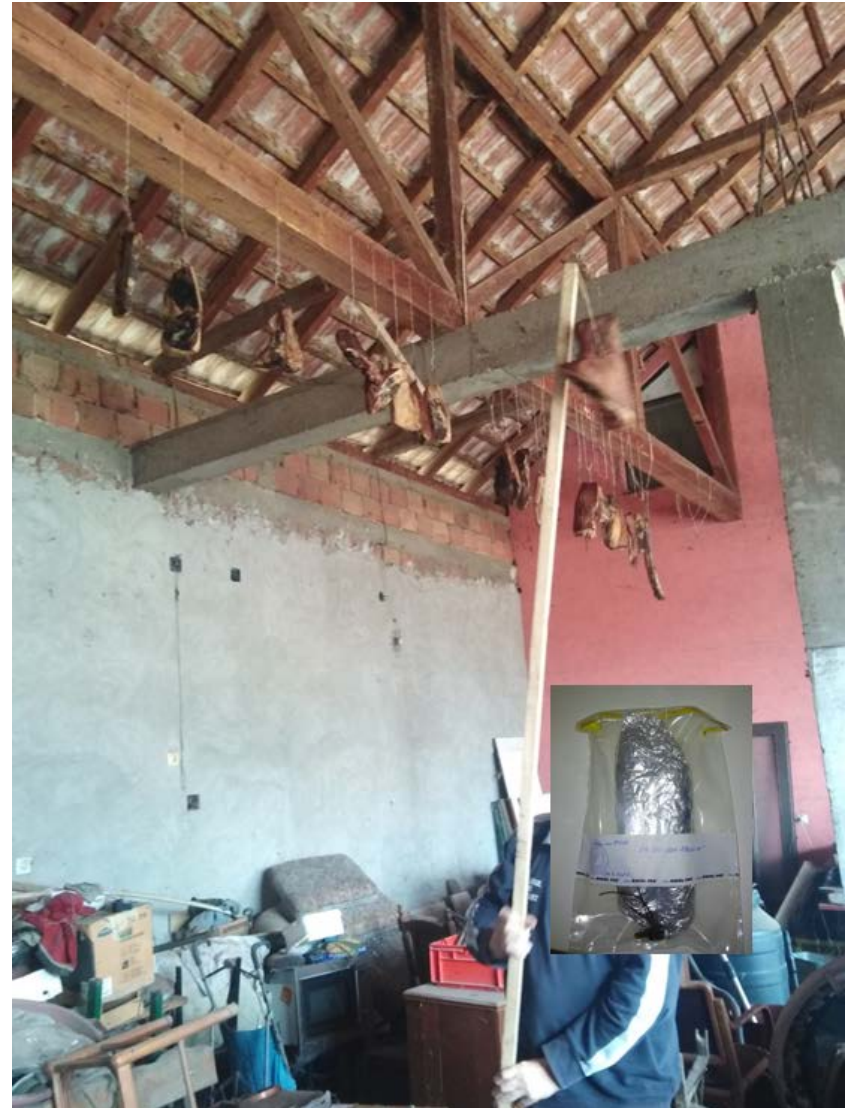
Trace back study performed by Veterinary Specialist Institute, Pozarevac

In December 2021, an inhabitant of village Pozezeno (M.S.), bought in the city Kladovo (District Branicevo) a ham of a wild boar from the hunters who stated that the meat was examined by veterinarian and safe for the consumption.

MS used this meat to prepare a “Kulen”. He, his wife and several friends (6 persons) tasted a Kulen at the end of January. Some other friends took it home as a gift and consumed it (15 persons) on the beginning of February. Finally, the same man organized a gathering of 20 friends at February 13 (7 patients reported consumption until mid-part of February).

The veterinary inspection visited his place on March 9, 2022. The owner stated that there were no more Kulen in his possession. All Kulen samples given to other households were confiscated and *Trichinella* larvae were detected by VSI Pozarevac specialist.

In collaboration with EURLP, ISS, Rome, Italy, *Trichinella spiralis* was identified (7 LPG).



Outbreak of Trichinellosis in Pozezeno, March 2022

Trace back study performed by epidemiologists and NRLT-INEP engagement

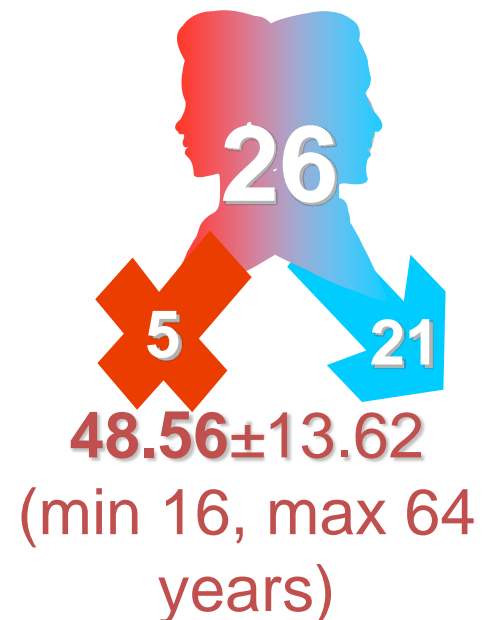
The first patient with symptoms of trichinellosis contacted the medical doctor on February 10 (at app. 3 weeks p.i.). A total of 28 patients suspected on trichinellosis were examined until March 15. There were no inpatients due to the mild clinical picture and lack of complications.

NRLT-INEP was contacted at March 9 by Ministry of Health, Institute of Public Health of Serbia and local institutions. Patient's sera collection and transfer were organized at March 22 and April 6 (the first blood sample) by the **Institute of Public Health of Pozarevac**.

Based on the analysis of *T. spiralis* specific antibody presence in the first as well as in the second blood sample (April 18, samples for At and cell analysis) the **diagnosis of trichinellosis was confirmed by NRLT-INEP in 26/28 patients.**



Ivana Mitic and Lj. Sofronic, contact with patients from Pozezeno, filling out questionnaires, taking blood samples in **Community Health Center Veliko Gradiste**



Outbreak of Trichinellosis in Pozezeno, March 2022

Clinical and Laboratory findings

Fifteen patients out of total 26 with confirmed trichinellosis **provided a consent to participate in this study** (at app. 10 weeks p.i.) (Gender and age are presented at the Figure).

Clinical and Laboratory findings

Myalgia (100%),

Fever (66.7%),

Weakness (40%),

Eyelid edema (40%),

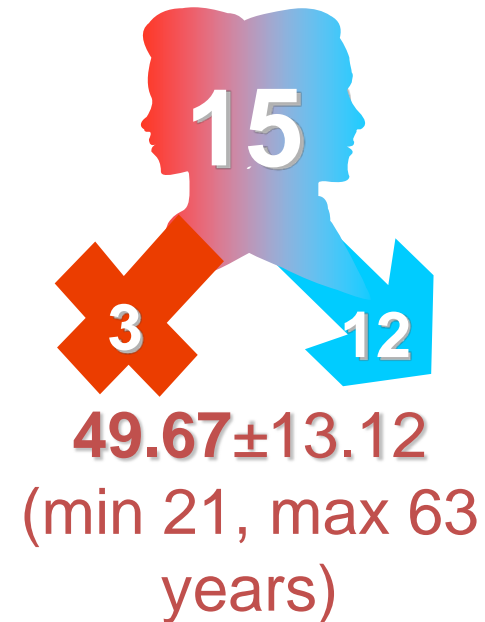
Nausea/diarrhea (33.3%),

Arthralgia (26.7%)

Headache (6.7%).

Eosinophil count 12.3-34.6 %

Elevated CK (U/L) 211-484 (5 patients)



Outbreak of Trichinellosis in Pozezeno, March 2022

The investigation of the impact of infection with *T. spiralis* to the HUMORAL immune response against the parasite itself and the SARS-CoV-2 virus

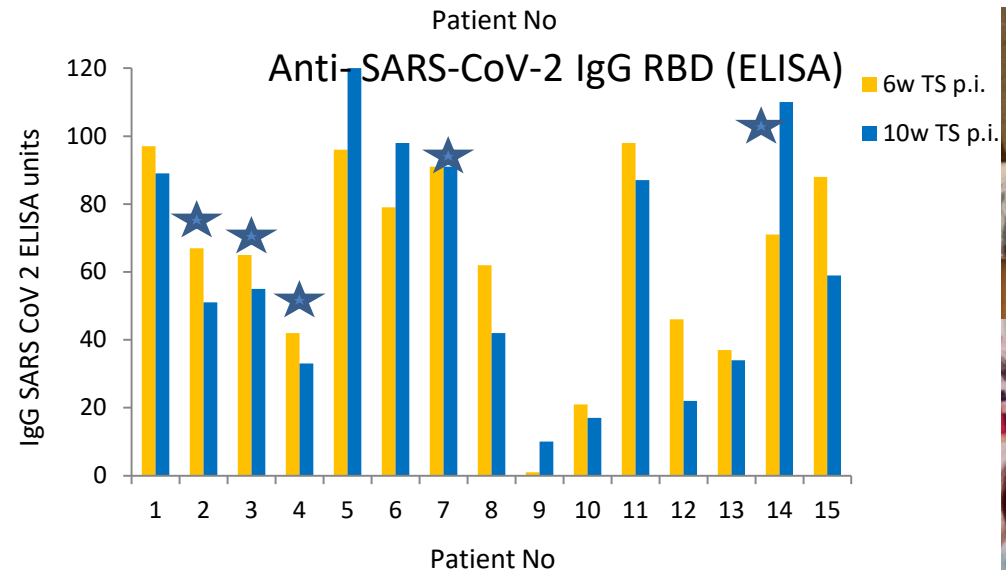
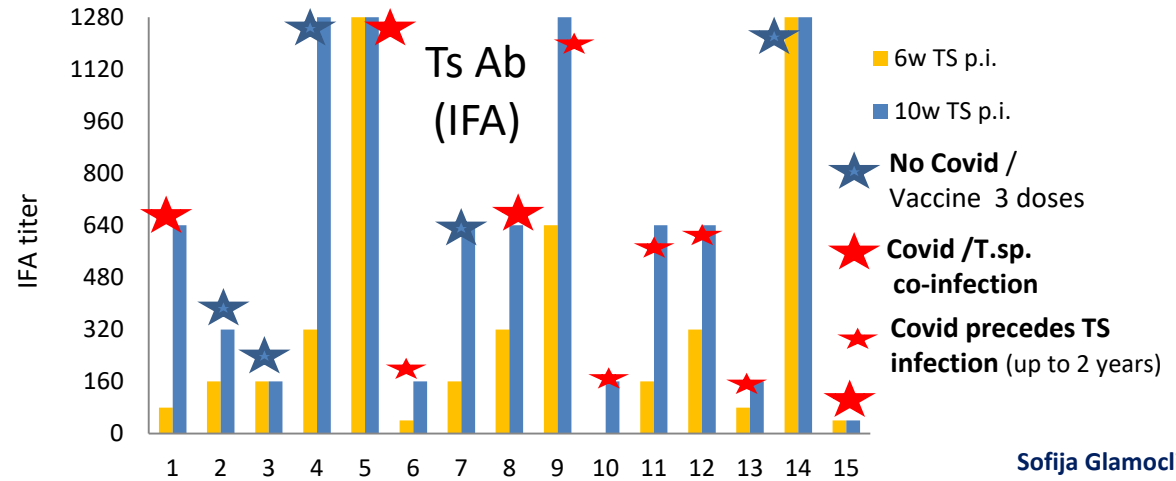
At NRLT INEP

The presence of **anti-*Trichinella* antibodies**, by IFA („FITC *Trichinella spiralis* Antibody Detection Kit, INEP, Serbia“)

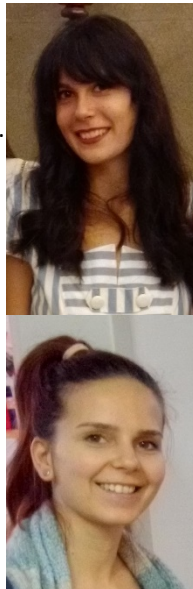
The presence of **anti- SARS-CoV-2 antibodies**, by ELISA („ELISA SARS-CoV-2 IgG (RBD – S protein), INEP, Serbia“)

Ten patients had an infection with the SARS-CoV-2 virus, the rest were fully immunized.

All 15 patients had both types of antibodies. No significant changes in antibody presence specific for helminth as well as for Covid-19 infection was detected in second (10w. p.i.) compared to first blood sampling (6w. p.i.).



Sofija Glamoclija,
Ljiljana Sabljic



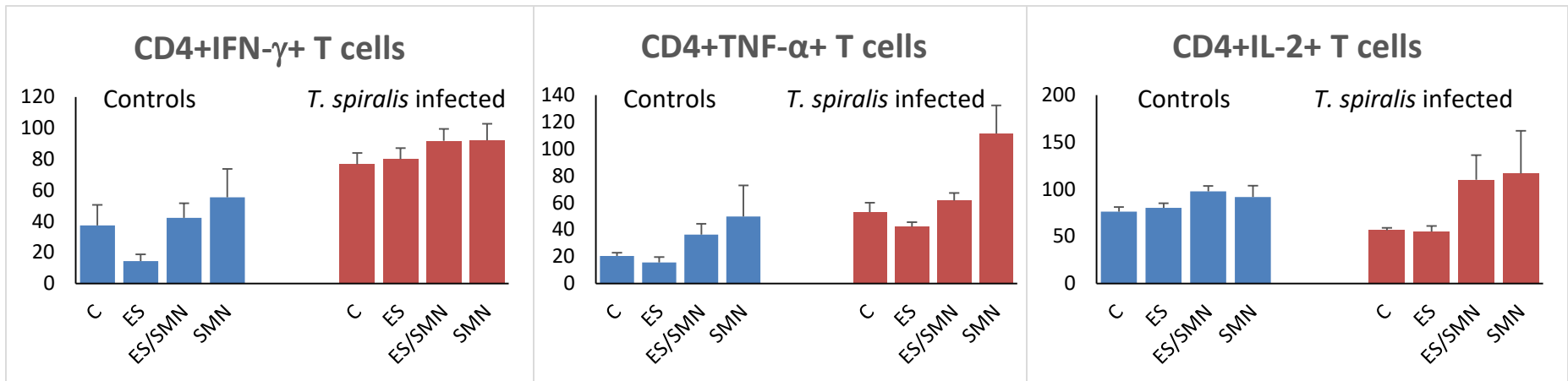
Outbreak of Trichinellosis in Pozezeno, March 2022

The investigation of the impact of infection with *T. spiralis* to the T CELL RESPONSE on *T. spiralis* ES and the SARS-CoV-2 antigens



N. Ilic and A. Gruden Movsesijan

Th1 TYPE OF IMMUNE RESPONSE



The expression of Th1 and Th2 cytokines was investigated in CD4+ T cells using flow cytometry.

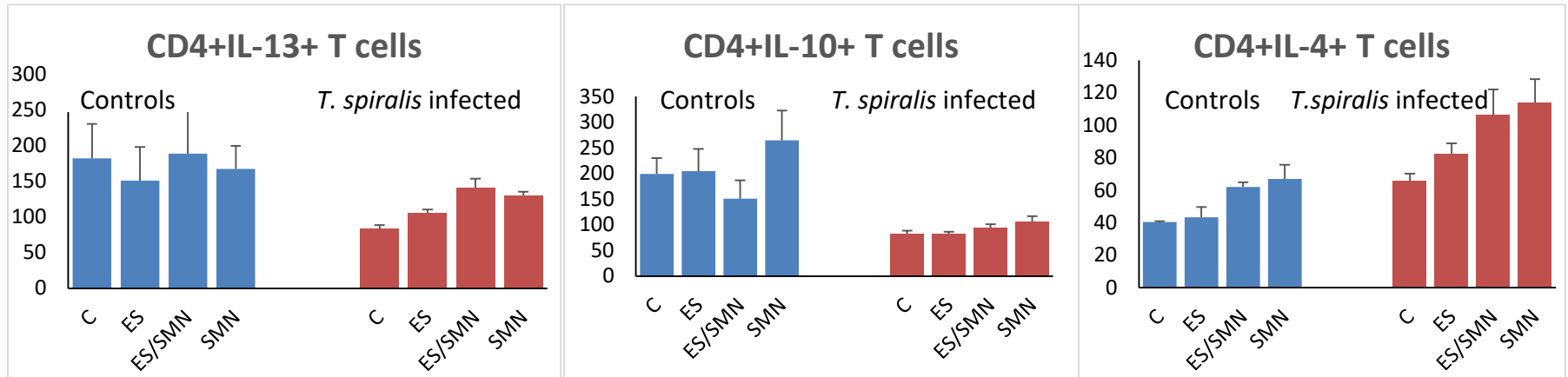
Here we monitored the **antigen specific immune response** using *T. Spiralis* (*TS*) excretory-secretory products (ES) and SARS-CoV-2 peptides (S, M, N), as well as the impact of parasite products on the reactivity of T cells to virus peptides (ES/SMN), 10 w. TS p.i. **All 15 patients** were included in this type of analyses (due to the small study group). Controls – healthy persons who were fully vaccinated and/or recovered from Covid-19.

Results – Th1 type of immune response was detected by stimulation of TS specific effector T ly in response to ES antigens. *Trichinella* infection did not influence the capacity of patient's effector T cells (specific for SARS-CoV-2) to produce IFN- γ , TNF- α and IL-2 upon stimulation with SMN antigens i.e. ***Trichinella* does not provoke suppression of Th1 type of immune response to virus antigens.**

Outbreak of Trichinellosis in Pozezeno, March 2022

The investigation of the impact of infection with *T. spiralis* to the T CELL RESPONSE on *T. spiralis* ES and the SARS-CoV-2 antigens

Th2 TYPE OF IMMUNE RESPONSE



Results – Th2 type of immune response was detected by stimulation of TS specific effector T ly in response to ES antigens (most profound effect on IL-4 production).

Capacity of trichinellosis patient's effector T cells to respond to ES antigens by producing Th2 cytokines was not impaired by SARS-CoV-2 infection and/or vaccination or vice versa (No inhibition of SARS-CoV-2 reactive Th2 type of CD4+ by *T. spiralis* ES).

- ❑ Despite the good implementation of preventive measures by the veterinary services **in District Branicevo, the risk of an outbreak appearance** - due to consumption of uninspected, raw or undercooked meat or meat products prepared from domestic or wild boars - **is not negligible,**
- ❑ Looking for an answer to the question of whether *Trichinella* suppresses the immune response to viral infections (or vaccines), based on this small study we can consider that the answer is NO - it does not.
- ❑ Namely, ***Trichinella* infection in humans suppresses neither antibody production nor competent cellular response to SARS-CoV-2 virus antigens** (whether they originate from infections or vaccines).
- ❑ To the best of our knowledge, this is the first study that has been conducted in humans where the influence of trichinellosis to immune response to viral antigens was monitored.

Acknowledgement

- ❖ Mitic I., Ilic N., Gruden-Movsesijan A., Glamoclija S., Sabljic Lj. National Reference Laboratory for Trichinellosis. Department for immunology and immunoparasitology, **Institute for the Application of Nuclear Energy - INEP**, University of Belgrade, Belgrade, Serbia
- ❖ Zivojinovic M. **Veterinary Specialist Institute, Pozarevac**, Serbia
- ❖ Plavska D, Saponjic V. Department for Control and Prevention of Communicable Diseases, **Institute of Public Health of Serbia “Milan Jovanovic Batut”**, Belgrade, Serbia
- ❖ Stankovic N., **Institute of Public Health of Pozarevac**, Serbia.
- ❖ Stevanovic L., Vasic N., **Community Health Center Veliko Gradiste**, Serbia
- ❖ **EURLP, ISS, Rome, Italy**

ICT-16

16th International Conference on Trichinellosis

30.08.-01.09.2023, Belgrade, Serbia

WELCOME



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