

Serbian external quality assessment for *Trichinella* detection in meat in 2021 compared to 2017

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Abstract: In 2021, the Serbian National Reference Laboratory for Trichinellosis, Serbian Institute for the Application of Nuclear Energy, organized external quality assessment (EQA 2021) for the detection of *Trichinella* larvae presence in meat by the magnetic stirrer method (MSM). The aims of this study were to examine the performance of the accredited laboratories over time and to compare the participants' performance. EQA 2021 was organized according to ISO 17043, and the test panel consisted of three meat balls, two of which were spiked with four *Trichinella spiralis* L1 larvae. Evaluation of the qualitative results showed that 90.91% (100% in 2017) of participants successfully passed the EQA. Quantitative evaluation showed that, on average, 71.59% (in 2017 only 60%) of the spiked *Trichinella* larvae were detected. This study enabled comparisons of laboratories over time (2017 and 2021) and across the country. The results obtained should serve as motivation for improvement of laboratory performance. All official laboratories with accredited MSM for *Trichinella* detection should participate in an EQA every second year and all other laboratories that perform *Trichinella* testing should participate annually in EQAs organized at national level. Regular participation will bring improvement in sensitivity of the test method used and will promote the important one health concept.

Keywords: *Trichinella*, external quality assessment 2021, meat.

Introduction

Zoonotic foodborne parasites are important human health hazards. One of these pathogens is the helminth *Trichinella* (Pozio, 2020). Parasites of the genus *Trichinella* are widespread across the world, and domestic and wild animals can be infected (Pozio and Zarlenga, 2013; Noeckler et al., 2019). Humans are infected after consumption of meat containing viable *Trichinella* larvae (Pozio et al., 2003; Mayer-Scholl et al., 2017; Noeckler et al., 2019).

Examination of susceptible animals, especially pigs, is the main method for preventing trichinellosis in humans. In order to protect the health of European Union meat consumers, the Commission Regulation (EU) No. 2015/1375 (European Union, 2015) proclaims the rules for control of *Trichinella* in meat, and the magnetic stirrer method (MSM) for pooled sample digestion is stated as a reference method (Mayer-Scholl et al., 2017). All the laboratories that perform official controls for *Trichinella* presence in meat need to participate regularly in comparative inter-laboratory or proficiency testing (PT) (European Union, 2017).

In EU member states, the National Reference Laboratories for *Trichinella* provide PTs to

laboratories in order to evaluate the quality, competence and performance of the laboratory tests (Marucci et al., 2009; Riehn et al., 2013; Marucci et al., 2016). The Serbian National Reference Laboratory for Trichinellosis organized successfully in 2017 the first Serbian external quality assessment (EQA) for the detection of *Trichinella* larvae in meat for accredited laboratories only (Vasilev et al., 2019). Bearing in mind that Serbia is in the process of harmonizing its regulations with the EU, the regulations regarding the EQA should also agree with EU regulations. Therefore, all official, accredited laboratories for *Trichinella* detection need to participate in national PTs with the valuable aims of comparing their own performance with others and of improving their competence. As the next step towards these goals, the *Trichinella* EQA 2021 was recently conducted.

Materials and methods

Animals and parasite. *Trichinella spiralis* (strain ISS 161) was maintained at the Institute for the Application of Nuclear Energy (INEP), and the animal use complied with national regulations and institutional policies. Use of animals was approved by the Veterinary

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EQA. As described in detail in *Vasilev et al.* (2019), the EQA for detecting the presence of *Trichinella* larvae in meat was organized according to ISO 17043 (ISO/IEC 17043, 2010). The *Trichinella* EQA 2021 was announced to accredited laboratories early in 2021. We offered the laboratories the opportunity to assess their own performance through a confidential system of testing the samples and to determine their ability to perform the analytical procedure. The laboratory's aim within EQA 2021 was to correctly identify each sample in a test panel as *Trichinella*-positive or -negative. The EQA 2021 test panel consisted of three samples with identical numbers of viable *Trichinella* larvae for the participants. The procedure for preparation of EQA samples was described by *Marucci et al.* (2016) and *Vasilev et al.* (2019). Briefly, the test panel consisted of three meat balls, two of which were spiked with four viable *Trichinella spiralis* L1 larvae, while one meatball was not spiked (negative control). Larvae were obtained by digestion of *T. spiralis* infected rat carcasses. Larvae were counted under a stereo-microscope and transferred individually to each meat ball. The glass was examined twice

under the stereo-microscope to ensure that no larvae remained untransferred. Every single meat ball was enclosed in a bag and sealed under vacuum. For each participant laboratory, one coded envelope containing the three meat balls was then stored in a refrigerator until forwarding. EQA 2021 packages were forwarded the same day by courier in a thermo box containing ice packs with the aim to maintain a temperature of 4–15°C during the transport. The ice packs were separated from the meat balls by separators to avoid direct contact. All participating laboratories were invited to fill in forms to provide information about the package content and its condition at the moment of opening.

To check the test panel stability over time, one EQA test panel was stored at room temperature and tested by staff at INEP two days later.

The MSM for pooled sample digestion was used by all participants to analyse the EQA 2021 test panel of three meat balls (European Union, 2015).

Criteria for the result evaluation. According to European regulation (European Union, 2015), *Trichinella* evaluation is qualitative, so the task was to identify the samples in EQA 2021 as positive if *Trichinella* larvae were present or negative if larvae were not found. Final evaluation for the participant



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EPK je organizovan u skladu sa zahtevima standarda ISO 17043

IZVEŠTAJ TRICHINELLA EPK 2021

Ukupni rezultati učesća u eksternoj proceni kvaliteta (EPK) dijagnostike prisustva larvi *Trichinella spiralis* u mesu primenom metode magnetne mešalice

Dizajn		
Svrha	Provera kvaliteta rada laboratorija koja pregledaju meso na prisustvo larvi <i>Trichinella</i>	
Tip PT šeme	Pojedinačno, istovremeno više učesnika	
Učesnici	Laboratorije	
Broj učesnika	Zavisni od broja zahteva	
Metod	Metod veštačke digestije pomoću magnetne mešalice	
EPK uzorak	Matriks	Svinjsko meso
	Test materijal	<i>Trichinella</i> žive larve
	Broj uzoraka	Tri za svakog učesnika
	Distribucija	Odmah posle pripreme
Evaluacija rezultata	Kvalitativna	

Realizacija	
Broj učesnika	11
NIV	2
VSI	8
Druge laboratorije	1
Datum slanja	06.04.2021.
Datum slanja dodatnog panela	01.06.2021.

EPK uzorci	Svinjsko meso (100 grama svakog)	Ukupan broj distribuiranih uzoraka
Sastav EPK panela	2 pozitivna sa po 4 larve, 1 negativan	36
Sastav dodatnog EPK panela	2 pozitivna sa po 4 i 5 larvi, 1 negativan	
Kurir	Post expres	

Organizator EPK
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Analiza rezultata

U skladu sa direktivama EU predviđeno je da se rezultati veštačke digestije iskazuju samo kvalitativno, tj kao pozitivan ako su larve *Trichinella* prisutne u uzorku ili negativan ako larvi nema u uzorku. Za uspešan rezultat učešća potrebno je da svi pozitivni i negativni uzorci budu ispravno određeni.

Oznaka laboratorije	Broj uzoraka ispravno identifikovanih	Broj uzoraka koji NISU ispravno identifikovani	Rezultat učešća
1	3	0	USPEŠNO
2	3	0	USPEŠNO
3	3	0	USPEŠNO
4	3	0	USPEŠNO
5	3	0	USPEŠNO
6	1	2	NEUSPEŠNO
7	3	0	USPEŠNO
8	3	0	USPEŠNO
9	3	0	USPEŠNO
10	3	0	USPEŠNO
11	3	0	USPEŠNO
6*	3	0	USPEŠNO

*Ponavljanje testa posle sprovedenih odgovarajućih korektivnih mera

Rezultati zbirno

Broj laboratorija koje su učestvovalе	11 +1 *Ponavljanje testa
Broj učesnika koji su uspešno prošli EPK	10 +1 *Ponavljanje testa
Broj učesnika koji nisu prošli EPK	1 + 0 *Ponavljanje testa

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Figure 1. Example of final evaluation report supplied to laboratories participating in *Trichinella* EQA 2021.

was positive if all samples in EQA 2021 test panel were properly identified.

Quantitative evaluation, with the number of spiked and recovered L1 larvae for all samples, was also conducted. To compare the results between the EQA 2021 participants, the number of recovered larvae was presented as a percentage of spiked number of larvae in the sample (detected/spiked × 100).

EQA report. Each participant laboratory was provided with an EQA report. The overall summary of participant performance in *Trichinella* EQA 2021 contained only anonymized laboratory codes to guarantee confidentiality (Figure 1).

Results and discussion

Participation in *Trichinella* EQA 2021 remained on a voluntary basis. A total of 11 (from 13) accredited laboratories that cover the epizootiological area across the country (two Scientific Veterinary Institutes, located in Novi Sad and Belgrade, the Laboratory of the Food Testing Center (Centar za ispitivanje namirnica — CIN) and eight Veterinary Specialist Institutes located in the cities of Subotica, Pozarevac, Zajecar, Jagodina, Nis, Sombor, Kraljevo, Zrenjanin) agreed to participate (Figure 2). All the packages (11) with the EQA samples (33)

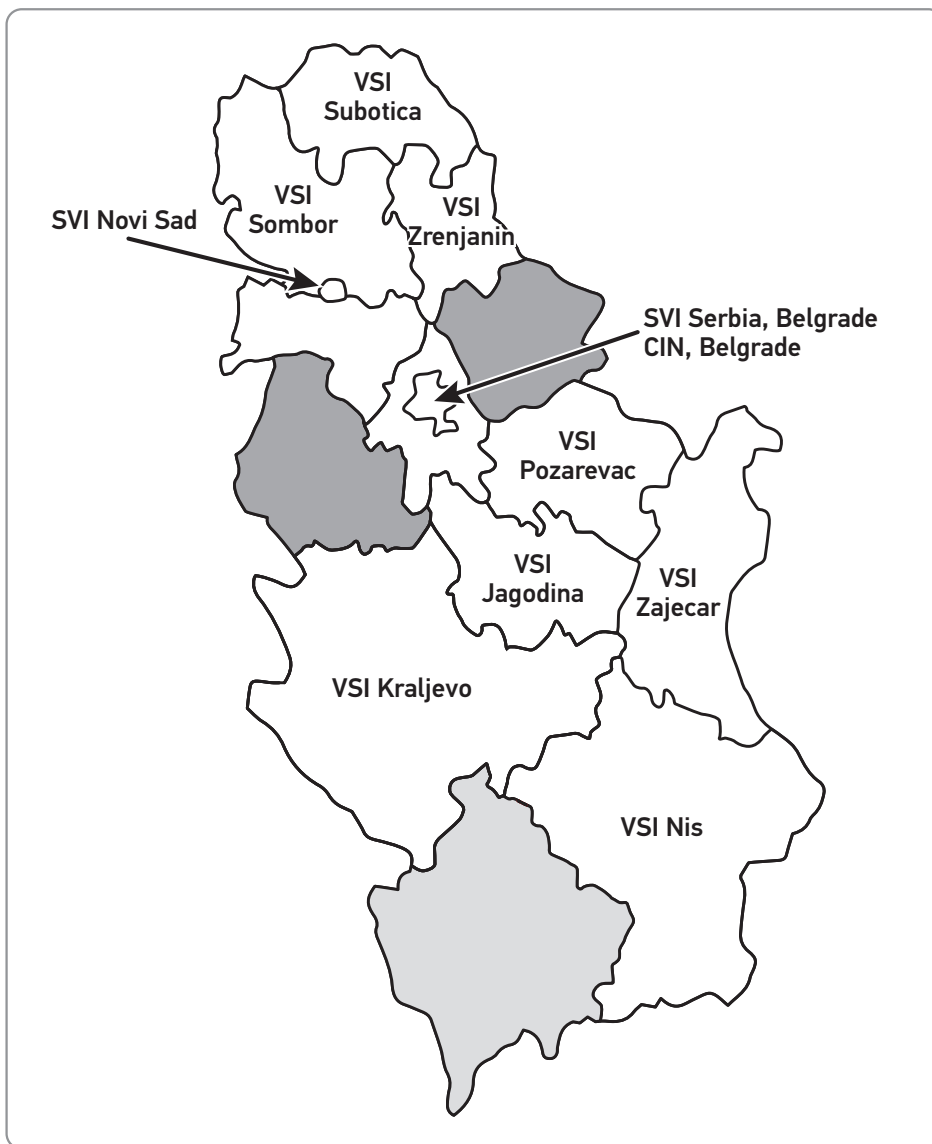


Figure 2. Epizootiological area in Serbia covered by participants in the EQA 2021 for *Trichinella* larvae detection

Legend: White – Epizootiological area covered by the Veterinary Specialist Institutes (VSIs) that participated in EQA 2021 (the name of the institute corresponds to the name of the city in which it is located). Dark grey – epizootiological area covered by a VSI that did not participate in EQA 2021. Light grey – Kosovo; no data. Note: The Scientific Veterinary Institute (SVI) Serbia, located in Belgrade, is a scientific institution that covers the whole territory of Serbia, while SVI Novi Sad, located in Novi Sad, is a scientific institution that covers the territory of Vojvodina province.

were delivered within 24 h to the participating laboratories. At the time of delivery, the internal temperature of all the packages was less than 15°C. Each participating laboratory performed controls on the package's content within 1 h of package arrival.

Qualitative results. One laboratory (with one false negative and one false positive result) did not pass the *Trichinella* EQA 2021. Ten of 11 (90.91%) participating laboratories passed the *Trichinella* EQA 2021 (Table 1). Analysis of the data obtained

from the first two Serbian *Trichinella* EQAs, conducted in 2017 and the current one in 2021, showed that the ability of the participants to classify test samples as true-positive or true-negative was satisfactory (Table 2). The specificity of the test method depends on the analyst's skills, and the results were satisfactory except for one accredited laboratory. The overall rate of correct analysis by the participants in 2017 was 100%, but in 2021, it was 93.94%. This is in agreement with the results achieved in the

Table 1. EQA 2021. Qualitative and quantitative results

Lab code	Number of larvae		Difference	Results	Final Evaluation
	spiked	found			
1	0	0	0	positive	positive
	4	3	1	positive	
	4	3	1	positive	
2	0	0	0	positive	positive
	4	3	3	positive	
	4	4	1	positive	
3	0	0	0	positive	positive
	4	3	1	positive	
	4	2	2	positive	
4	0	0	0	positive	positive
	4	4	0	positive	
	4	3	1	positive	
5	0	0	0	positive	positive
	4	4	0	positive	
	4	4	0	positive	
6	0	3	3	negative	negative
	4	0	4	negative	
	4	3	1	positive	
7	0	0	0	positive	positive
	4	2	2	positive	
	4	2	2	positive	
8	0	0	0	positive	positive
	4	3	1	positive	
	4	3	1	positive	
9	0	0	0	positive	positive
	4	3	1	positive	
	4	1	3	positive	
10	0	0	0	positive	positive
	4	1	3	positive	
	4	3	1	positive	
11	0	0	0	positive	positive
	4	3	1	positive	
	4	3	1	positive	
6*	0	0	0	positive	positive
	5	3	2	positive	
	4	3	1	positive	

*Repetition of the EQA after in-house analysis and applied corrective measures

Table 2. Comparison of participation in EQA 2017 and EQA 2021

Laboratory code	EQA 2017	EQA 2021
1	positive	positive
2	positive	positive
3	positive	positive
4	positive	positive
5	positive	positive
6	positive	negative
		positive*
7	positive	positive
8	positive	positive
9		positive
10		positive
11		positive

*Repetition of the EQA after in-house analysis and applied corrective measures

PTs organized by the European Union Reference Laboratory for Parasites (EURLP) for the period 2007–2021. The percentage of EURLP participants which passed the PT successfully for the mentioned period varied between 83.3% and 100% (Marucci et al., 2016; EURLP web site).

Quantitative results. In the *Trichinella* EQA 2021, one of the participant laboratories successfully detected all of the spiked L1 larvae. Figure 3 presents the percentage of larvae detected in the first two Serbian EQAs. Three laboratories were very successful (laboratories 2, 4 and 5) since the level of L1 larvae detection was 87.5 %, 87.5% and 100 % respectively. For laboratories 1, 8 and 11, the level of L1 larvae detection was 75%. Laboratories 7, 9 and 10 successfully detected 50% of the L1 larvae, while laboratory 6 detected 37.5% of the L1 larvae spiked into the meat balls, with one false negative and one false positive result, resulting in this laboratory failing the EQA. Interestingly, among the three laboratories that detected 50% of the spiked L1 larvae, there were clear differences in the number of larvae recovered from the individual meat ball samples. Laboratory 7 detected 50% of the larvae in

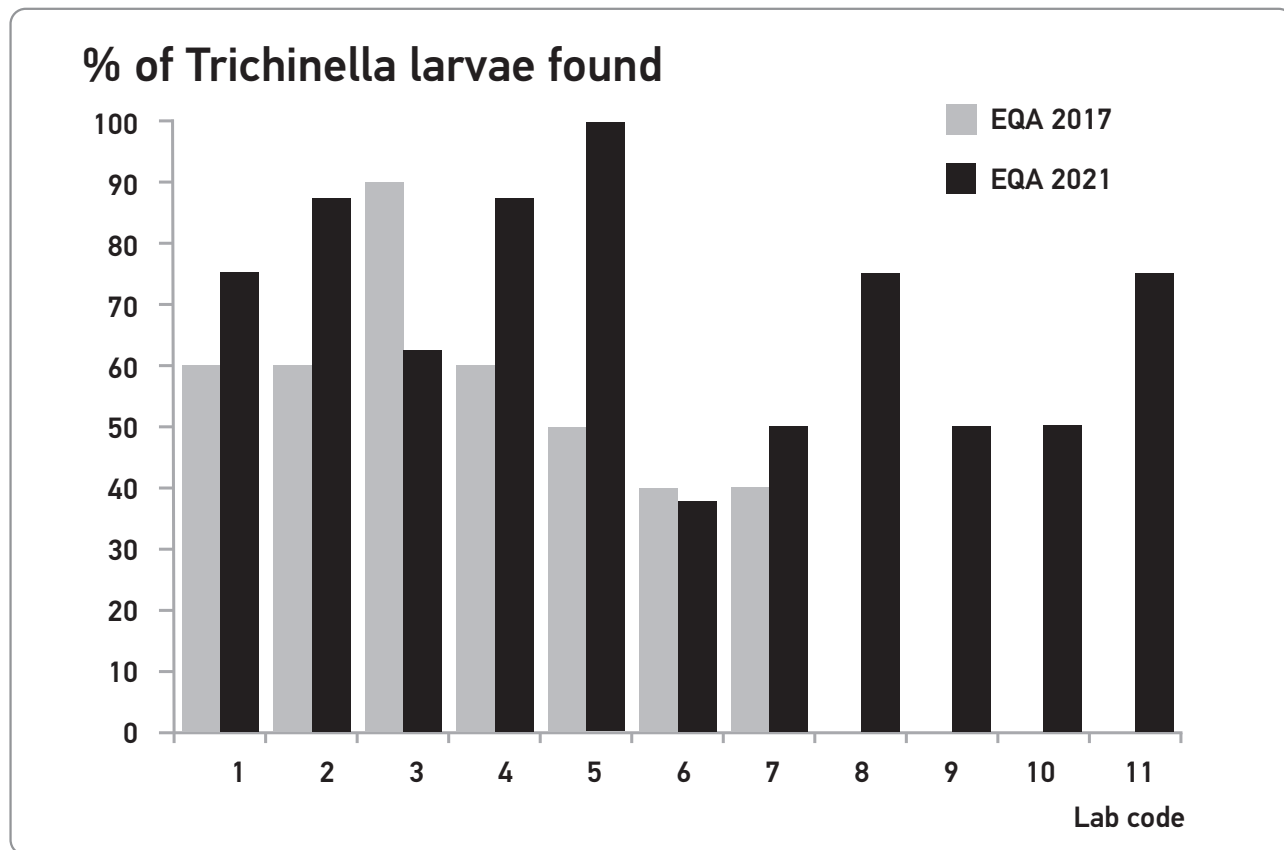


Figure 3. Quantitative results for the participating laboratories in *Trichinella* EQA 2017 and EQA 2021 in Serbia. Values are expressed as percentage of larvae detected in relation to the total number of larvae spiked per meat panel. One meat panel containing multiple samples was examined per year.

each sample, while laboratories 9 and 10 detected 75% of the larvae in one sample and 25% from another sample. Therefore, laboratories 9 and 10 were close to obtaining a false negative result (in one sample they detected only one larva from the four that were spiked). Marucci *et al.* (2016) showed that for the quantitative evaluation of samples containing less than six larvae, the Z-score should not be used. According to that, results with at least two larvae recovered should be acceptable for test samples with 4–5 larvae. If this criterion was applied to our *Trichinella* EQA 2021, laboratory 7 would meet the requirements (by finding two larvae of the four spiked, 50%), while laboratories 9 and 10 would not.

The finding of only one larva in samples with 4–5 L1 larvae initiates revision of laboratory standard operation procedure and monitoring of both the critical control points and the performance of the analyst. Comparing EQA 2017 with EQA 2021 indicated the performance of the laboratories is now worse than before, because in 2017, one laboratory had to apply in-house analysis after the EQA, but in 2021, three laboratories had to analyse their participation (one failed the EQA, and two found only one of four larvae in one sample). After in-house analyses and checking their critical control points, laboratory 6 requested a second EQA test panel. This panel consisted of two positive samples (meat balls with four and five larvae spiked) and one negative sample. Evaluation of results showed that this laboratory did then pass the requested test, with all three samples correctly identified and 75% of the larvae recovered.

The overall success in MSM performance on average was 60% in 2017, but in 2021 it was improved, with 71.59% of the larvae recovered. Quantitative results obtained in Serbian EQAs for *Trichinella* larvae detection (in 2017, 40–90% and in 2021, 50–100% depending on the participating laboratory) were similar to results from EU PTs (Marucci *et al.*, 2009; Marucci *et al.*, 2016). The current results are also similar to results from German PTs when laboratories found 60% of the L1 larvae (Riehn *et al.*, 2013). Marucci *et al.* (2016) and Riehn *et al.* (2013) showed that participating in PTs year by year improves laboratory performance (it is considered as good performance if more than 80% of larvae are recovered, and there are no false negative or false positive results). In relation to this, the results obtained by EQA 2021 should be considered as improvement of the MSM performance over time in the participating laboratories. The goal that laboratories should try to achieve could be set to 50% or more larvae recovery from each individual sample.

It is important to keep in mind the critical control points for the MSM are a way to improve laboratory performance (Djordjevic *et al.*, 2013; Mayer-Scholl *et al.*, 2017; ICT guidelines). Detection of fewer L1 larvae than were spiked could be explained by errors related to critical control points (in each step 2–3% of the larvae are lost) (Riehn *et al.*, 2013).

Before our *Trichinella* EQA, some Veterinary Specialist Institutes participated in PTs organized by different EU providers. For laboratories in Serbia, these PTs have many disadvantages, especially long transport, very high prices and inability to compare performances (personal communication). We participated in 11 PTs organized by EURLP from 2009 to 2021 and successfully passed them all (even when samples were spiked with 1 larva). The National Reference Laboratory for Trichinellosis in INEP has been a member of the EURLP Network from 2008. In *Trichinella* EQA 2021, the criterion for positive evaluation of participants was the correct detection of *Trichinella* presence or absence in the sample. The numbers of spiked larvae in EQA 2021 samples was similar to the numbers spiked by EURLP after 2015 (Marucci *et al.*, 2016). According to Forbes *et al.* (1998), in the test panel samples, one larva can be detected by artificial digestion, but a limit of three larvae in PT samples for participating routine laboratories is more appropriate. With that knowledge, we decided to spike the samples with four larvae. The results of *Trichinella* EQAs clearly confirm our capability as PT provider to conduct this very important activity, as well as the knowledge and skills of participants.

In Serbia, *T. spiralis* infected pork is usually the source of *Trichinella* infection (Sofronic-Milosavljevic *et al.*, 2013, NRLT unpublished data). In last ten years, the rate of pig infection significantly decreased from 0.02% to 0.003% (unpublished data) because of improvements in pig production, better control measures and public education. In Serbia, the sources of outbreaks are usually meat from untested infected backyard pigs (Sofronic-Milosavljevic *et al.*, 2013, Vasilev *et al.*, 2019) and, rarely, wild boars (Pavic *et al.*, 2020). Also, testing for *Trichinella* larvae in meat is regularly performed in laboratories of the Scientific Veterinary Institutes, Veterinary Specialist Institutes, veterinary hospitals, veterinary practices and slaughterhouses. However, the EQA in 2017 and especially the current one in 2021 (with one laboratory that failed to fulfil requirements) pointed to the need for training personnel in official laboratories for *Trichinella* detection, control of equipment used and regular participation in national PTs.

Conclusion

Ten of 11 participants successfully passed the Serbian *Trichinella* EQA 2021. These EQA results demonstrated the overall good performance of participants, but at the same time pointed to the need for training, control of equipment and regular participation in national PTs. An important feature regarding this study is that it enabled comparison of

participant performance over time and also with the anonymized results of the other accredited Serbian *Trichinella* testing laboratories. The current study strongly indicates the need for all *Trichinella* laboratories in Serbia to participate annually in EQAs organized at national level. Regular participation could improve their performance, provide valuable and useful data for the Veterinary Directorate, and be of help in promoting the one health concept.

Eksterna provera kvaliteta otkrivanja prisustva larvi *trichinella* u mesu u Srbiji u 2021 i poređenje sa 2017

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Apstrakt: U 2021 godini Nacionalna referentna laboratorija za trihinelozu (NRLT) je organizovala eksternu proveru kvaliteta (EPK) za otkrivanje larvi trihinela u mesu korišćenjem metode magnetne mešalice za zbirni uzorak (MMM). Cilj je bio da se omogući zainteresovanim akreditovanim laboratorijama da uporede kvalitet rada tokom vremena, ali i uporede svoj rezultat sa drugim učesnicima. EPK je organizovan prema standardu ISO 17043. Test panel poslat u 11 laboratorija se sastojao od po 3 loptice od mesa sa identičnim brojem larvi. U dva uzorka bilo je dodato po četiri živih mišićnih L1 larvi *Trichinella spiralis* a jedan uzorak je bio bez larvi. Procena kvalitativnih rezultata je pokazala da je 90,91% (2017 je bilo 100%) učesnika uspešno prošlo testiranje. Prosecan prinos larvi iznosio je 71,59% (dok je 2017 bilo 60%). Ovaj rad je po prvi put omogućio poredjenje rezultata laboratorija tokom vremena, ali i poređenje između laboratorija. Učešće u EPK treba da posluži za poboljšanje izvodjenja metode magnetne mešalice za zbirni uzorak. Zato bi bilo poželjno da ove ali i druge laboratorije u Srbiji učestvuju u narednim EPK ili PT šemama organizovanim na nacionalnom nivou. Cilj je da ove aktivnosti dovedu do poboljšanja senzitivnosti metode, pruže korisne informacije kolegama ali i Upravi za veterinu i da budu od značaja za promociju koncepta „jedno zdravlje“.

Ključne reči: reč, *Trichinella*, eksterna procena kvaliteta, svinjsko meso.

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