

## TRICHINELLOSIS IN SERBIA AND POSSIBILITIES TO IMPROVE CONTROL MEASURES

ŽIVOJINOVIĆ Milena<sup>1</sup>, DOBROSAVLJEVIĆ Ivan<sup>1</sup>, KULIŠIĆ Zoran<sup>2</sup>,  
RADOJIČIĆ Sonja<sup>2</sup>, BOŠKOVIĆ Tamara<sup>3</sup>, PLAVŠIĆ Budimir<sup>4</sup>

<sup>1</sup>Veterinary Specialistic Institute Pozarevac, Serbia; <sup>2</sup>University of Belgrade, Faculty of Veterinary Medicine, Belgrade, Serbia; <sup>3</sup>Ministry of Agriculture, Forestry and Water Management, Veterinary Directorate, Serbia; <sup>4</sup>World Organization for Animal Health (OIE), OIE Regional Representation for Europe (Moscow)

Received 12 April 2019; Accepted 08 June 2019  
Published online: 11 October 2019

Copyright © 2019 Živojinović et al. This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

**How to cite:** Živojinović Milena, Dobrosavljević Ivan, Kulišić Zoran, Radojičić Sonja, Bošković Tamara, Plavšić Budimir. Trichinellosis in Serbia and the possibility of improvement to control measures. *Veterinarski Glasnik*, 2019. 73 (2), 108-115. <https://doi.org/10.2298/VETGL190412018Z>

### Abstract

**Background.** Trichinellosis is zoonotic disease caused by parasites of the genus *Trichinella*, which have a cosmopolitan distribution. In the Balkan region, including Serbia, trichinellosis is endemic. Although trichinellosis shows a decreasing incidence in Serbia, this disease remains a serious problem for human health and animal husbandry.

**Scope and Approach.** To prevent and control *Trichinella* spp. infections in domestic and sylvatic animals, risk analysis for these zoonotic parasites should be conducted. In Serbia, a limited number of *Trichinella* have been identified to the species level so far. An institutional, multi-sectoral surveillance system, supported by an adequate legal framework for the detection, surveillance, prevention, control and reporting of this infection in animals and humans, harmonised with the EU legislation, is a priority.

**Key Findings and Conclusions.** One of the main goals of the Serbian veterinary and public health services should be progressive improvement of animal rearing practices, food safety, and hunters' and consumers' education through the "One-health" approach.

**Key Words:** *Trichinella*, control, risk factors, animal, health

---

\*Corresponding author – e-mail: [povetinst\\_milenaz@hotmail.com](mailto:povetinst_milenaz@hotmail.com)

## INTRODUCTION

Epizootiological investigations have confirmed that zoonotic parasites of the genus *Trichinella* are still a serious problem for human health and animal husbandry in Serbia (Zivojinovic et al., 2013). In the Balkan region, *Trichinella* spp. infections are endemic (Balić et al., 2015; Boutsini et al., 2014; Urošević et al., 2013; Blaga et al., 2009; Zivojinovic et al., 2009, Cuperlovic et al., 2005). The occurrence of human infections is mainly due to the presence of *Trichinella* spp. in backyard and free-ranging pigs and wildlife (Dmitric et al., 2018; IPHS, 2018; Boutsini et al., 2014; Zivojinovic et al., 2013).

Official diagnostic methods to detect *Trichinella* larvae in muscles of domestic pigs intended for human consumption are the trichinoscopy method and artificial digestion of muscle tissues. For detection in meat of wild boars and horses, artificial digestion is the mandatory diagnostic method (Official Gazette of the Republic of Serbia (RS) for the current year). At present, Serbian legislation includes the *Law on Veterinary Matters* (Official Gazette RS 91/2005, 30/2010 and 93/2012) and regulations based on the *Food Safety Law* (Official Gazette RS 41/2009). As a part of the twinning project *Enhancing the capacities of Serbian authorities in zoonoses and food borne disease control Serbia* (Twinning reference SR 13 IB AG 01), the Veterinary Directorate, as the national competent authority, in partnership with the Ministry of Health, and with the support of Veterinary Institutes are making efforts in improving animal and human health, including further alignment of the Serbian legislation with the European Union legislation.

### **Trichinella infection in Serbia**

So far, surveillance and monitoring of *Trichinella* spp. in domestic (pigs and dogs) and wild animals (wild boar, *Sus scrofa*; red foxes, *Vulpes vulpes*; golden jackals, *Canis aureus* and; wolves, *Canis lupus*) was carried out in some endemic districts in Serbia (Klun et al., 2019; Dmitrić et al., 2017; Ćirović et al., 2015; Teodorović et al., 2014; Zivojinovic, 2013; Zivojinovic et al., 2010; Zivojinovic et al., 2009). An investigation was also focused on the transmission pathways of *Trichinella* species (Živojinovic et al., 2015).

In Serbia, *T. spiralis* is the most frequent *Trichinella* species detected in domestic pigs and wild boars, whereas *T. britovi* was more frequently documented in wild carnivores (Zivojinovic 2013), and was detected in only one domestic pig and one wild boar in Zlatibor and Pirot districts (Cvetkovic et al., 2011). Mixed infections with *T. spiralis* and *T. britovi* were documented in three carnivores (Zivojinovic, 2013). In Serbia, *Trichinella* larvae have never been documented in slaughtered horses; however, *T. spiralis* larvae were detected in horses imported from Serbia to France and Italy (Pozio et al, 2001), where they caused human outbreaks of trichinellosis (Pozio, 2015). *T. spiralis* and *T. britovi* are the two main species circulating in Europe (Pozio et al., 2009) and the Balkan countries bordering Serbia (Blaga et al., 2007). *T. pseudospiralis* has been documented in

domestic pigs in Croatia and Bosnia and Herzegovina (Santrac et al., 2015; Beck et al., 2009), but there is still no available data for Serbia.

Trichinellosis is one of the most important zoonosis in Braničevo District. The re-emergence of trichinellosis was officially recognised in 1999, and in 2003, the local government declared six parishes as endemic *Trichinella* areas and established control measures (Zivojinovic et al., 2009). In the course of an epidemiological survey on *Trichinella* spp. circulating in Braničevo District (Zivojinovic et al. 2010), muscle samples from domestic and wild animals were collected and tested by the artificial digestion method to collect *Trichinella* larvae according to the Commission Regulation (EC) No. 2075/2005 and the Manual of Standards for diagnostic tests and vaccines for terrestrial animals of the World Health Organization for Animal Health (European Commission, 2005; OIE, 2013). After digestion, larvae were washed 3-5 times in cold water, counted in triplicate and fixed in absolute ethyl alcohol for molecular identification at the species level by multiplex PCR at the International *Trichinella* Reference Center (ITRC), Rome, Italy, according to a validated protocol (<http://www.iss.it/crlp>). Geographical data of the *Trichinella* isolates from Braničevo District were collected using GARMIN Map Source (Garmin, Taiwan), and *Trichinella* isolates were mapped (Zivojinovic et al. 2010). These data allowed the identification of the endemic foci in the district.

### **Risk factors and control measures**

The most important risk factors for the establishment of the *Trichinella* domestic cycle are: (a) the intentional feeding of food waste containing pork scraps or intentional or unintentional exposure to carcasses of dead swine or wildlife; these risks are usually encountered in free-range pasturing; (b) allowing pigs to scavenge on garbage dumps; (c) using wild game carcasses or scraps from hunting as animal feed; (d) feeding horses with pork scraps or with carcasses of fur animals; (e) using carcasses of slaughtered for animals as feed for other fur animals present on the farm (Gamble et al., 2000; Pozio & Murrell, 2006; Pozio, 2014).

In Serbia, the finding of *T. spiralis* in domestic pigs and in hunted wild boars near human settlements could be explained by the behaviour of raising pigs near rivers, small waterways and ponds where they can come into contact with wildlife (Zivojinovic et al., 2013). Additionally, free-ranging pigs and stray dogs are not prevented from scavenging on garbage dumps (Zivojinovic et al., 2010). The husbandry conditions of 90% of backyard farms with *Trichinella* infection are very poor (Zivojinovic et al., 2010). In Serbia, the practices of feeding pigs with food waste of home or restaurant origin that can contain raw pork, scavenging of pigs in garbage dumps and the improper disposing of pig carcasses in the field are on-going (Zivojinovic et al., 2013). These inappropriate rearing conditions of domestic pigs favour wild animals feeding on pork scraps and offal and on pig carcasses.

Pig meat samples can be examined by trichinostomy and/or artificial digestion at licentiate veterinary services according to official regulations (Official Gazette RS for the current year). However, the low sensitivity of trichinostomy as a diagnostic method and the possibility of missing non-encapsulated *Trichinella* (Forbes et al. 2003) contribute to less reliable data about prevalences of *Trichinella* spp. in Serbia. Through harmonisation of a strategic, institutional and legal framework for detection, surveillance, prevention, control and reporting of zoonoses, Serbia will be able to develop *Trichinella* control measures in compliance with the EU requirements. Further efforts should focus on harmonising with the EU requirements, improving hygienic conditions on farms, expanding systematic meat inspection of domestic pigs and wild boars and proceeding with risk communication on trichinellosis between veterinary services, farm owners and hunters.

In the case of a positive finding, information should be submitted to veterinary inspectors. According to the Serbian veterinary regulations, it is mandatory to report *Trichinella* findings, because of their zoonotic potential, to the national veterinary authority (Plavšić et al., 2009). All data are entered in the Animal Notification System (VetUP software application) and become available to all responsible services. Awareness amongst stakeholders and the general public of potentially zoonotic *Trichinella* infection (control activities, prevention, EU standards and requirements etc.) should be increased. The identification of *Trichinella*-infected animals allows identification of the foci of transmission, so veterinary services, the owners of pig farms and slaughterhouses and hunter's associations can be informed about the risk of transmission of these zoonotic agents. An awareness-raising campaign should be implemented in the whole country, particularly in risk areas. Improving biosecurity on animal farms, promoting prevention measures and implementing good farming practices will certainly improve animal health, human health and environmental protection in Serbia.

From 1968 to 2018, at the International *Trichinella* Reference Center (<https://trichinella.iss.it/>) 79 *Trichinella* isolates from Serbia were identified as follows: (a) *Trichinella spiralis*, 62 isolates from domestic pigs (*Sus scrofa domestica*), domestic horses (*Equus ferus caballus*), wild boars (*Sus scrofa*), red foxes (*Vulpes vulpes*), wolves (*Canis lupus*), golden jackals (*Canis aureus*), wild cat (*Felis silvestris*) and stray dogs (*Canis lupus familiaris*); (b) *Trichinella britovi*, 14 isolates from red foxes (*Vulpes vulpes*), wolves (*Canis lupus*) and golden jackals (*Canis aureus*); and (c) *T. spiralis* and *T. britovi* mixed infections in two red foxes and one golden jackal.

In the last 100 years in Europe, the increase in forests and fallow land, concomitant with a decrease in farms, has favoured the expansion of wild boar (*Sus scrofa*) populations and, consequently, increased *Trichinella* transmission among animals and humans. The common habits of hunters of leaving animal carcasses in the field after skinning or removing and discarding the entrails increases the probability of *Trichinella* transmission to new hosts (Pozio & Murrell, 2006).

According to OIE data and the Institute of Public Health of Serbia (IPHS, 2018), 15 human *Trichinella* infections in Serbia were documented in 2017, showing a decrease of this zoonosis in the last ten years when the yearly incidence was between 46 and 190 infections. The decrease of human trichinellosis in Serbia suggests the efforts of veterinary and public health services are producing positive results.

## CONCLUSION

As a part of the twinning project *Enhancing the capacities of the Serbian authorities in zoonoses and food borne disease control Serbia* (Twinning reference SR 13 IB AG 01) Serbia is making an effort to improve animal and human health. Only through harmonisation of a strategic, institutional and legal framework for detection, surveillance, prevention, control and reporting of zoonoses, Serbia will be able to be in compliance with EU requirements. An improvement of the professional capacity of the competent authorities to implement and enforce standards and regulations for the control of *Trichinella* infections in animals and humans is expected.

## Authors contributions

MZ designed the paper, selected references for the presentation and wrote manuscript. ID assisted in data collection and analysis. ZK made substantial contribution to the conception and interpretation of data. SR made critical revise of the concept, gave substantial contribution to analysis and interpretation and was involved in drafting the manuscript and revising critically. TB assisted in data collection and provided legislative framework. BP made substantial contribution to the conception, acquisition and interpretation of data.

## Competing interests

The authors declare that they have no competing interests.

## REFERENCES

- Balić D., Marinculić A., Krešić A., Barić J., Periškić M., Škrivanko M., Kovač Z., Krznarić M. l. 2015. Working group for trichinellosis – a way of systematic prevention, control and eradication of trichinellosis in the republic of Croatia. *Collegium Antropologicum*, 39 (1): 253–259.
- Beck R., Beck A., Lucinger S., Florijancic T., Boskovic I., Marinculic A. 2009. *Trichinella pseudospiralis* in pig from Croatia. *Veterinary Parasitology*, 159: 304–307. doi:<https://dx.doi.org/10.1016/j.vetpar.2008.10.067>
- Blaga R., Durand B., Antoniu S., Gherman C., Cretu C.M., Cozma V., Boireau, P. 2007. A dramatic increase in the incidence of human trichinellosis in Romania over the past 25 years: impact of political changes and regional food habits. *The American Journal of Tropical Medicine and Hygiene*, 76: 983-986.

- Blaga R., Gherman C., Cozma V., Zocevic A., Pozio E., Boireau P. 2009. *Trichinella* species circulating among wild and domestic animals in Romania. *Veterinary Parasitology*, 159: 218–221. doi: <https://doi.org/10.1016/j.vetpar.2008.10.034>
- Boutsini S., Papatsiros V. G., Stougiou D., Marucci G., Liandris E., Athanasiou L. V., Papadoudis A., Karagiozopoulos E., Biasis A., Pozio E. 2014. Emerging *Trichinella britovi* infections in free ranging pigs of Greece. *Veterinary Parasitology*, 199 (3–4): 278–282. <https://doi.org/10.1016/j.vetpar.2013.10.007>
- Ćirović D., Teodorović V., Vasilev D., Marković M., Ćosić N., Dimitrijević M., Klun I., Djurković-Djaković O. 2015. A large-scale study of the *Trichinella* genus in the golden jackal (*Canis aureus*) population in Serbia. *Veterinary Parasitology*, 212 (3–4): 253–256. doi: <https://doi.org/10.1016/j.vetpar.2015.07.022>
- Cuperlovic K., Djordjevic M., Pavlovic S. 2005. Re-emergence of trichinellosis in southeastern Europe due to political and economic changes. *Veterinary Parasitology*, 132: 159–166. doi: <https://doi.org/10.1016/j.vetpar.2005.05.047>
- Cvetkovic, J., Teodorovic, V., Marucci, G., Vasilev, D., Vasilev, S., Cirovic, D., Sofronic-Milosavljevic, Lj. 2011. First report of *Trichinella britovi* in Serbia. *Acta Parasitologica*. 56: 232–235. doi: <https://doi.org/10.2478/s11686-011-0022-1>
- Dmitric M., Debeljak Z., Vidanovic D., Sekler M., Vasković N., Matović K., Karabasil N. 2018. *Trichinella britovi* in game meat linked to human trichinellosis outbreak in Serbia. *Journal of Parasitology*, 104(5):557–559. <https://doi.org/10.1645/18-42>
- Dmitric M., Vidanovic D., Vaskovic N., Matovic K., Sekler M., Debeljak Z., Karabasil N. 2017. *Trichinella* infections in red foxes (*Vulpes vulpes*) and golden jackals (*Canis aureus*) in six districts of Serbia. *Journal of Zoo and Wildlife Medicine*, 48(3):703–707. doi: <https://doi.org/10.1638/2016-0169.1>
- European Union. 2005. Commission Regulation (EC) No. 2075/2005 of the European Parliament and of the Council of 5 December 2005 laying down specific rules on official controls for *Trichinella* in meat. *Official Journal of the European Union*, L 338: 60–82.
- Forbes L. B., Parker S., Scandrett W. B. 2003. Comparison of a modified digestion assay with trichinoscopy for the detection of *Trichinella* larvae in pork. *Journal of Food Protection* 66: 1043–1046
- Gamble H. R., Bessonov A. S., Cuperlovic K., Gajadhar A. A., van Knapen F., Nockler K., Schenone H., Zhu X. 2000. International Commission on Trichinellosis: recommendations on methods for the control of *Trichinella* in domestic and wild animals intended for human consumption. *Veterinary Parasitology*, 93: 393–408.
- IPHS. 2018. The report on infectious diseases in Serbia in 2017. Institute of Public Health of Serbia, Belgrade, Serbia, p 49. Available at: <http://www.batut.org.rs/download/izvestaji/Godisnji%20izvestaj%20zarazne%20bolesti%202017.pdf>
- Klun I., Ćosić N., Ćirović D., Vasilev D., Teodorović V., Djurković-Djaković O. 2019. *Trichinella* spp. in wild mesocarnivores in an endemic setting. *Acta Veterinaria Hungarica*, 67 (1): 34–39. <https://doi.org/10.1556/004.2019.004>
- Law on Food Safety. Official Gazette, RS No 41/09.
- Law on Veterinary Matters. Official Gazette SCG No 91/2005, 30/2010 and 93/2012.
- Manual of Diagnostic Tests and Vaccines for Terrestrial Animals OIE, 2018, Chapter 3.1.20 Trichinellosis (Infection with *Trichinella* spp.), 649–659
- Plavsic B., Nedic D., Micovic Z., Stanojevic S., Asanin R., Krnjajic D., Tajdic N., Milanovic S. 2009. Veterinary information management system (VIMS) in the process of notification

- and management of animal diseases. Acta Vet-Beograd, 59: 99-108. doi: <https://doi.org/10.2298/AVB0901099P>
- Pozio E. 2007. Taxonomy, biology and epidemiology of *Trichinella* parasites. Guidelines for the surveillance, management, prevention and control of trichinellosis, FAO/WHO/OIE, 31-35.
- Pozio E. 2015. *Trichinella* spp. imported with live animals and meat. Veterinary Parasitology, 213 (1–2): 46-55.
- Pozio E., Hoberg E., La Rosa G., Zarlenga D. S. 2009. Molecular taxonomy, phylogeny and biogeography of nematodes belonging to the *Trichinella* genus. Infection, Genetics and Evolution, 9: 606-616. <https://doi.org/10.1016/j.meegid.2009.03.003>
- Pozio E., Murrell K. D. 2006. Systematics and epidemiology of *Trichinella*. Advances in Parasitology, 63: 368-439. [https://doi.org/10.1016/S0065-308X\(06\)63005-4](https://doi.org/10.1016/S0065-308X(06)63005-4)
- Pozio E., Tamburrini A., La Rosa G. 2001. Horse trichinellosis, an unresolved puzzle. Parasite, 8: 263-265. doi: <https://doi.org/10.1051/parasite/200108s2263>
- Santrac V., Nedic D., Maric J., Nikolic S., Stevanovic O., Vasilev S., Cvetkovic J., and Sofronic-Milosavljevic Lj. 2015. The first report of *Trichinella pseudospiralis* presence in domestic swine and *T. britovi* in wild boar in Bosnia and Herzegovina. Acta Parasitologica, 60(3): 471–475. doi:<https://dx.doi.org/10.1515/ap-2015-0066>.
- Teodorović V., Vasilev D., Ćirović D., Marković M., Ćosić N., Djurić S., Djurković-Djaković O. 2014. The Wolf (*Canis lupus*) as an indicator species for the sylvatic *Trichinella* cycle in the central Balkans. Journal of Wildlife Diseases, 50 (4): 911-915. <https://doi.org/10.7589/2013-12-333>
- Urošević M., Petrović J., Mirilović M., Ristić Z., Jajić I. 2013. Characteristics of human trichinellosis in the territory of Vojvodina in the period 2002-2011. Arhiv veterinarske medicine, 6(1): 45-55.
- Zivojinovic M. 2013. Epizootiological, serological and molecular investigations of *Trichinella* species, PhD thesis, University of Belgrade, Faculty of Veterinary Medicine, Belgrade, Serbia
- Živojinović M., Sofronić Milosavljević Lj., Kulišić Z., Radojičić S. 2015. Risk factors in domestic and wild cycles of *Trichinella* species. *One Health – New Challenges* First International Symposium of Veterinary Medicine (ISVM 2015), 83
- Zivojinovic M., Sofronic-Milosavljevic Lj., Cvetkovic J., Pozio E., Interisano M., Plavsic B., Radojicic S., Kulisic Z. 2013. *Trichinella* infections in different host species of an endemic district of Serbia. Veterinary Parasitology, 194: 136-138. <https://dx.doi.org/10.1016/j.vetpar.2013.01.039>
- Zivojinovic M., Sofronic-Milosavljevic Lj., Radojicic S., Kulisic Z. 2010. Application of GIS in epizootiological surveillance of swine trichinellosis in one endemic district in Serbia. Parasite, 17, 369-373. <https://dx.doi.org/10.1051/parasite/2010174369>
- Zivojinovic, M., Dimitrijevic, G., Lazic, M., Petrovic, M., Sofronic Milosavljevic, Lj., 2009. *Trichinella* prevalence in swine in an endemic district in Serbia: epidemiology and control. Veterinary Parasitology, 59: 358–360. <https://dx.doi.org/10.1016/j.vetpar.2008.10.074>

## TRIHINELOZA U SRBIJI I MOGUĆNOST UNAPREĐENJA KONTROLNIH MERA

ŽIVOJINOVIĆ Milena, DOBROSAVLJEVIĆ Ivan, KULIŠIĆ Zoran,  
RADOJIČIĆ Sonja, BOŠKOVIĆ Tamara, PLAVŠIĆ Budimir

### Kratak sadržaj

*Uvod.* Trihineleza je zoonotska bolest uzrokovana parazitima roda *Trichinella* koji imaju kosmopolitsku rasprostranjenost. U regionu Balkana, uključujući Srbiju, trihineleza je endemska. Iako trihineleza pokazuje trend smanjenja učestalosti u Srbiji, ova bolest ostaje ozbiljan problem za zdravlje ljudi i stočarstvo.

*Cilj i pristup.* Za sprečavanje i kontrolu infekcije domaćih i silvatičnih životinja trihinelama, treba sprovesti analizu rizika za ove zoonotske parazite. U Srbiji je do sada identifikovan ograničeni broj trihinelama na nivou vrste. U tom smislu, prioritet je institucionalni, multisektorski sistem nadzora, podržan odgovarajućim pravnim okvirom za otkrivanje, nadzor, prevenciju, kontrolu i izveštavanje o ovoj infekciji kod životinja i ljudi, usklađen sa zakonodavstvom EU.

*Ključni nalazi i zaključak.* Jedan od glavnih ciljeva veterinarske i zdravstvene službe trebalo bi biti progresivno unapređenje prakse uzgoja životinja, bezbednost hrane i obrazovanja lovaca i potrošača kroz pristup „One-Health“.

**Ključne reči:** Trihinelama, kontrola, faktori rizika, životinje, zdravlje