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EPIDEMIOLOGY OF HUMAN TRICHINELLOSIS IN VOJVODINA PROVINCE, SERBIA, FROM 2005 TO 2016

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Trichinellosis is one of the most important foodborne diseases in the Eastern European countries. The main objective of this study was to investigate the epidemiological patterns of trichinellosis outbreaks that occurred between 2005 and 2016 in Vojvodina, a northern province of the Republic of Serbia. The average incidence was 3.5 per 100,000 inhabitants. A total of 828 people acquired the infection. The disease occurred in all age groups, slightly more often in males, and quite frequently in a severe form considering the high share of hospitalised patients and the fatal outcome rate (41.6 ± 31.1% and 0.4%, respectively). *Trichinella spiralis* was confirmed as the causative agent in eight outbreaks. The outbreaks usually occurred among family members due to the consumption of pork or traditional pork products from not tested backyard pigs. Veterinary control measures and the education of consumers and farmers should be implemented to control this zoonotic disease.

Key words: *Trichinella spiralis*, outbreaks, pork, backyard pig, Serbia

Trichinellosis is a globally widespread foodborne parasitic zoonosis (Pozio, 2007). Domestic and wild pigs serve as the major *Trichinella* reservoirs for human infection (Murrell and Pozio, 2011; EFSA, 2017). The only way of trichinellosis transmission is the consumption of raw or undercooked meat from *Trichinella*-infected animals (Pozio, 2015). Human trichinellosis is a serious zoonosis documented in 55 countries worldwide (Pozio, 2007). The incidence of human trichinellosis in the EU countries in 2016 was 0.02 per 100,000 population, with the majority of cases recorded in Bulgaria and Romania (0.49 and 0.13 per 100,000, respectively) (EFSA, 2017). Trichinellosis in Western Europe is usually rare. Contrary to this, trichinellosis occurs in the form of sporadic cases or epidemics every year in Poland, Slovakia, Romania, Bulgaria, Serbia, Croatia, and Bosnia (Caccio et al., 2018).

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Trichinella infections in humans and animals are mandatorily reported in the Republic of Serbia, a country with close to 7.2 million inhabitants, located in South-eastern Europe (Anonymous, 2016). The disease belongs to the most important foodborne zoonoses in the country due to the occurrence of large outbreaks, lethal outcomes and economic losses. The spread of *Trichinella* infections is largely influenced by the poor socioeconomic conditions, inadequate education of breeders, and reduced veterinary controls (Tešić et al., 2011; Petrović et al., 2014; Petrović et al., 2017).

Despite the mandatory inspection of meat from *Trichinella*-susceptible animals, a significant incidence of human trichinellosis is registered annually, particularly in the Northern Province of Vojvodina with a human population of about two million (IPHV, 2018). Massive pig breeding with many meat-processing facilities and the widespread production of traditional meat products in the households are common in this province. Therefore, the main objective of this study was to describe the epidemiological features of trichinellosis and to identify the critical points that led to the occurrence of the disease in Vojvodina in the period 2005–2016.

Materials and methods

Data sources and extraction

Notifications of human cases of trichinellosis reported in Vojvodina between 2005 and 2016 were extracted from the surveillance database of the Institute of Public Health (IPH) of Vojvodina (IPHV, 2018). Demographic data as well as data on disease onset date, hospitalisation, laboratory confirmation and fatal outcome were collected. Data on the number and epidemiological features of outbreaks were included in the analysis. Annual reports of the IPH of Serbia 'Dr Milan Jovanović Batut' were used as a data source (IPHS, 2018).

Results of laboratory testing for the presence of *Trichinella* larvae in meat and meat products sampled during outbreak investigations were collected from the database of the laboratory of the Scientific Veterinary Institute 'Novi Sad' (SVI) for the period 2005–2016. Trends in disease incidence, number and seasonality of outbreaks, data of patients and *Trichinella* species identified as the aetiological agent of human infections were analysed.

Molecular identification of Trichinella *species*

Samples (n = 37) of meat and meat products collected in the course of epidemiological investigations of eight outbreaks were tested for the presence of *Trichinella* larvae by artificial digestion according to the Commission Regulation EC No 2075/2005 (EC, 2005). Larvae from the positive samples (n = 14) were collected and tested by PCR. DNA was extracted by the 'QIAamp® DNA Mini Kit' (Qiagen, Germany), with fast 'spin-column' procedure for DNA isolation,

using the tissue protocol according to the manufacturer's instructions. The PCR reaction was performed using the HotStar Taq Master Mix Kit (Qiagen). PCR amplification was performed according to the Multiplex PCR protocol of the European Reference Laboratory for Parasites (EURLP) for the identification of *Trichinella* muscle stage larvae at the species level (EURLP, 2012). *Trichinella spiralis* and *T. britovi* larvae identified at the species level in the SVI laboratory and confirmed by EURLP, were used as reference larvae.

Statistics

The incidence of trichinellosis was calculated using IBM SPSS Statistics 20 (IBM, Armonk, NY, USA version: http://www-01.ibm.com/support/docview.wss?uid=swg215090 12). P values < 0.05 were considered significant. Trends of incidence data were measured by GSI Mann–Kendall Toolkit For Constituent Trend Analysis (version 1.0 November 2012). Mann–Kendall statistic (S) negative values indicate the disease incidence over time. The strength of trend is proportional to the magnitude of S. Confidence in trend of 90% represents a significant level for ά = 0.1 and 95% confidence corresponds to ά = 0.05 (Newell et al., 2007).

Results

Incidence of trichinellosis in Vojvodina

Throughout the investigated period, the average annual incidence of human trichinellosis in Serbia was 1.80 per a population of 100,000 people (Table 1). The average incidence in Vojvodina was slightly higher than that in the whole country and Central Serbia, but the difference was not statistically significant.

Between 2005 and 2016, a total of 828 people suffered from trichinellosis in Vojvodina (Table 2, Fig. 1). The diagnosis was based on clinical signs and symptoms (e.g., fever, muscle soreness and pain, diarrhoea, facial oedema, eosinophilia) and on the epidemiological link with infected meat. In rare cases the disease was confirmed by serology. In total, 55 outbreaks of trichinellosis, ranging from one to ten per year (average 4.6 ± 3.0), were reported. The average attack rate was 56.6 ± 27.6% (range 12.0–100%). The vast majority of patients (n = 768; 92.7%) were outbreak cases, while the other 60 patients were considered as single cases (Table 2). The average annual number of cases was 69 (range 9–277 cases). The highest incidence was recorded in 2005 (n = 277; 13.2/100,000 inhabitants) for the occurrence of eight outbreaks. Outbreaks affected mostly family members (n = 26) or members of two or more related families (n = 14).

Table 1

Incidence of trichinellosis per 100,000 inhabitants in Serbia from 2005 to 2016

Year	Serbia	Central Serbia	Vojvodina
2005	4.5	1.1	13.6
2006	2.5	1.7	4.9
2007	2.4	2.2	2.8
2008	1.2	0.7	2.8
2009	0.7	0.1	2.2
2010	1.5	1.9	0.5
2011	1.7	1.1	3.5
2012	0.6	0.7	0.57
2013	1.3	0.5	3.6
2014	1.2	0.9	1.9
2015	1.1	0.5	2.8
2016	2.7	2.7	2.7
Average	1.8	1.2	3.5
SD[a]	1.1	0.8	3.4
Min	0.6	0.1	0.5
Max	4.5	2.7	13.6
CV[b]	0.6	0.7	1.0
S[c]	–22	–8	–24
Confidence factor	92.4%	68.1%	94.2%
Incidence trend	Probably decreasing	Stable	Probably decreasing

a = standard deviation, b = coefficient of variation, c = Mann–Kendall statistics

Age and gender distribution

Although men made up the majority of patients (n = 481, 58.1%), there was no statistically significant difference between genders (P = 0.25). The age-specific incidence rates ranged from 22.5 (age 0–4 years) to 58.5 (age 10–14 years) per 100,000 people with no significant differences between age groups (P = 0.54–0.99) (Table 3).

Severity of the disease

Fatal outcomes due to trichinellosis were recorded only in 2005. Two deaths were due to acute myocarditis and endocarditis, while the third fatal case was an immunocompromised patient who had already been hospitalised for other reasons. The mortality incidence for trichinellosis in 2005 was 0.02 per 100,000 population in Serbia, 0.02 in Central Serbia and 0.10 in Vojvodina. The case fatality rate was 0.8% in Serbia, 1.6% in Central Serbia and 0.7% in Vojvodina. Between 2005 and 2016, the average share of hospitalised patients within the total number of patients with trichinellosis was 41.6 ± 31.1% (range 0–100%) per year.

Table 2

Outbreaks of trichinellosis in the Province of Vojvodina, Republic of Serbia, from 2005 to 2016

Year	Number of patients	Number of outbreaks	Number of patients affected in outbreaks	Share of patients affected in outbreaks (%)
2005	277	8	277	100.0
2006	98	6	80	81.6
2007	57	10	55	96.5
2008	55	4	51	92.7
2009	44	4	43	97.7
2010	10	2	8	80.0
2011	69	1	67	97.7
2012	9	1	4	44.4
2013	69	8	49	71.0
2014	36	1	34	94.4
2015	53	6	42	98.1
2016	51	4	48	94.1
Total	828	55	758	85.7
Average	69.0	4.6	63.2	85.0
SD[a]	69.9	3.1	70.7	15.9
Min	9	1	4	44.4
Max	277	10	277	100.0

a = standard deviation

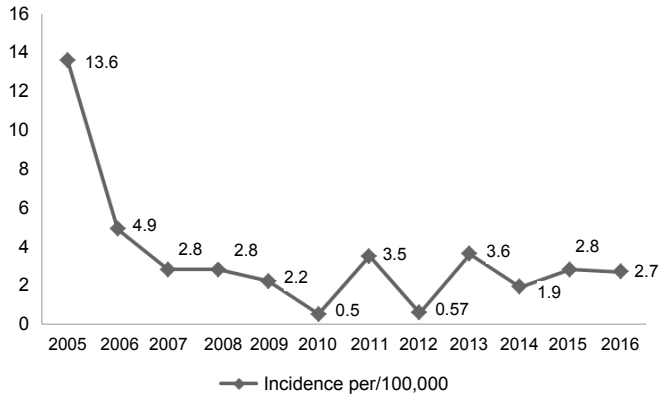

Fig. 1. Incidence of trichinellosis per 100,000 inhabitants in the Province of Vojvodina, Republic of Serbia from 2005 to 2016

Seasonality

The occurrence of trichinellosis in Vojvodina showed a strong seasonality ($P < 0.0049$). The majority of cases was recorded in the winter season (December–February, n = 35, 63.6%) (Fig. 2).

Table 3

Demographic features of patients with trichinellosis in the Province of Vojvodina. Republic of Serbia from 2005 to 2016

Age group	Number of cases by gender		Total	Age-specific incidence*
	Male	Female		
0–4	5	15	20	22.5
5–9	27	14	41	43.2
10–14	35	20	55	58.5
15–19	30	22	52	47.3
20–29	72	36	108	42.6
30–39	83	62	145	54.1
40–49	94	55	149	56.5
50–59	66	70	136	44.9
≥ 60	69	53	122	26.7
Total	**481**	**347**	**828**	**42.9**

*Age-specific rate is a rate for a specified age group, in which the numerator and denominator refer to the same age group (per 100,000 population)

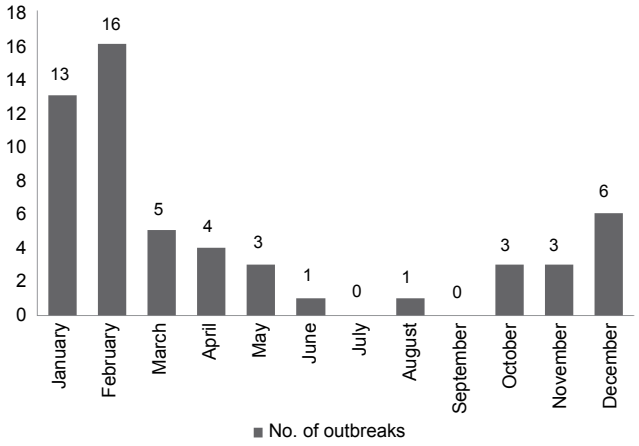

Fig. 2. Seasonality of trichinellosis outbreaks in the Province of Vojvodina, Republic of Serbia from 2005 to 2016

Molecular identification of Trichinella

Meat samples (n = 95; from 1 to 7 per outbreak) collected in the course of epidemiological investigations of 19 outbreaks (34.5%) were tested by artificial digestion. *Trichinella* larvae were collected in the course of 18 outbreak investigations. All *Trichinella* larvae isolated in the last eight outbreak investigations were identified as *T. spiralis* by PCR.

Incriminated food as a source of infection in the trichinellosis outbreaks

The consumption of meat and/or meat products from domestic pigs was the cause of all recorded outbreaks of trichinellosis. The most common incriminated food included traditional pork products (54.9%), pork from private slaughter (15.7%) or both (23.5%). The consumption of home-made raw sausages was identified as the source of infection for only one outbreak (5.9%).

Outbreak investigations

The cause of the large outbreaks with 103 cases in 2005 and 48 cases in 2006 was the consumption of traditional meat products purchased at the illegal market. In 2011, the outbreak with 67 cases was caused by the consumption of pork from a pig which had been tested by an unauthorised and untrained analyst. Not tested backyard pigs were the most common source of infection (54.9%). Illegally purchased meat products and mistakes in the meat inspection caused 33.3% and 11.8% of trichinellosis outbreaks in Vojvodina, respectively.

Discussion

In Vojvodina, trichinellosis has continuously been documented since 1966, when mandatory reporting was introduced. Massive outbreaks of trichinellosis occurred in the 1980s and 1990s (e.g., 907 human cases in 1985) (IPHV, 2018). Nevertheless, the incidence of trichinellosis shows a slightly decreasing trend both in Serbia and Vojvodina, most likely as the consequence of an increased awareness of the meat and meat product producers, the increasing number of properly farmed pigs and the reduction of uncontrolled backyard pigs. Throughout the study period, trichinellosis was reported with a higher incidence than other foodborne parasitic zoonoses such as echinococcosis and toxoplasmosis (Petrović et al., 2017), in the same way as in neighbouring Romania (Neghina et al., 2011). Mandatory systematic reporting of echinococcosis and toxoplasmosis in Serbia was initiated in 1964 and 1991, respectively (IPHV, 2018).

Trichinella spiralis is highly infective for domestic pigs and rodents and, luckily, its larvae in pork do not survive freezing (Murrell et al., 2000; Pozio, 2007). *Trichinella spiralis* is more pathogenic to humans than other *Trichinella* species, probably due to the higher proliferation of larvae as compared to those of *T. britovi* (Pozio et al., 1993).

Only *T. spiralis* and *T. britovi* have been reported so far in animals of Serbia (Živojinović et al., 2013). *Trichinella spiralis* is the only species confirmed up to now in domestic and wild swine in Vojvodina (Petrović et al., 2014). *Trichinella britovi* has recently been identified as the causative agent of a large outbreak in Central Serbia (Caccio et al., 2018). In the neighbouring countries, *T.*

spiralis is the most frequent species in swine, whereas *T. britovi* is more frequently documented in carnivorous mammals, while *T. pseudospiralis* was rarely documented (Neghina et al., 2008; Pozio, 2016). Trichinellosis is significantly more frequently recorded in the period between December and February compared to the rest of the year in Vojvodina as well in the whole of Serbia and in most countries of Europe, Asia and America, due to the practice of household slaughtering of pigs, when traditional meat products are made in the cold season (Wang et al., 1998; Ribicich et al., 2005; Neghina et al., 2008; Kurdova-Mintcheva et al., 2009; Ofori-Belić et al., 2010).

Trichinellosis is recorded in all age groups in Vojvodina; however, as expected, the incidence was the lowest in children up to 4 years, since they consume small quantities of traditional meat products that have strong salty taste (Petrović et al., 2014). In the present study, the incidence of trichinellosis in the age group of 10–14 years was the highest among the other age groups. This high incidence could be due just to chance, since at the international level trichinellosis occurs more frequently among persons of 20–50 years of age (median 33.1 years) (Murrell and Pozio, 2011).

Trichinellosis has a high illness attack rate (12–100%) in Vojvodina. The timely diagnosis of trichinellosis is a challenge due to the non-specific clinical picture at the initial stage; the symptoms are similar to those of influenza: weakness, headache, myalgia, oedema, fever, stomach pain, and diarrhoea (Kociecka, 2000). In addition to laboratory features (e.g. eosinophilia), epidemiological links such as the consumption of *Trichinella*-infected meat and/or a link to patients with trichinellosis have an important role in the diagnosis of this disease (Dupouy-Camet et al., 2002).

The clinical manifestation of the disease depends on the *Trichinella* species, the number of infective larvae ingested and the immune status of the patient (Capo and Despommier, 1996; Dupouy-Camet et al., 2002). Severe complications of acute trichinellosis are myocarditis, endocarditis, encephalitis, and meningitis (Dupouy-Camet et al., 2002). Complications usually have a poor prognosis and may lead to a fatal outcome. The following complications of trichinellosis were documented in 16% of hospitalised patients in Serbia: myocarditis-pericarditis, pneumonia and neurological symptoms (confusion, disorientation, headache) (Ofori-Belić et al., 2010). The hospitalisation rate of patients with trichinellosis (41.6 ± 31.1%) in Vojvodina was much higher than that in Belgrade (13.5%), the capital city of Serbia, in the period 2001–2008 (Ofori-Belić et al., 2010), which may be the result of different hospitalisation criteria rather than the disease severity.

Fatal outcomes usually occur between the third and fifth week of infection (Capo and Despommier, 1996). In Vojvodina, deaths occurred as a result of severe complications or due to immunodeficiency. The case fatality rate of trichinellosis in Serbia in the studied period was 0.4%; this value is higher than the

average case fatality rate (0.2%) detected at the world level from 1986 to 2009 (Murrell and Pozio, 2011). In the course of trichinellosis, death is rare, but the fatal outcome emphasises the importance to control *Trichinella* infections in susceptible animals to prevent trichinellosis in humans.

The most common foods incriminated in the trichinellosis outbreaks of Vojvodina, in the same way as in Romania and Bulgaria, are traditional meat products originating from backyard pigs. Although these meat products differ among these three countries, they are usually homemade and consumed undercooked (Kurdova-Mintcheva et al., 2009; Neghina et al., 2011). In Vojvodina, traditional meat products (ham, fermented and grill sausages) that are subject to drying and/or smoking, are a common source of *Trichinella* infection for humans. The consumption of this traditional food is characterised by insufficient thermal treatment to kill *Trichinella* larvae. In the course of epidemiological investigations of trichinellosis outbreaks, it is difficult to identify which meat or meat product may have been the source of infection, because the family usually had consumed different meals and meat-derived products following household slaughtering.

Even if the most frequent cause of the emergence of trichinellosis outbreaks was the consumption of meat from a not inspected pig (in 87.2% of patients), in a number of cases (12.8% of patients) outbreaks were caused by incorrectly tested pigs or wild boar such as: *(i) Trichinella* test performed by a not trained analyst; *(ii)* use of an insufficiently sensitive diagnostic test such as the compressorium method (trichinoscope) instead of the more sensitive artificial digestion test; *(iii)* inadequate sampling, e.g. examination of the intercostal muscles instead of the diaphragm by the compression method; *(iv)* insufficient sample size, e.g., examination of less than 10 g of wild boar diaphragm and less than 2 g of domestic pig diaphragm by artificial digestion (Commission Regulation 2015/1375).

In conclusion, trichinellosis is one of the major food-borne zoonoses continuously reported in Vojvodina. The disease can be severe up to fatal outcomes, with high attack and hospitalisation rates. The consumption of raw or undercooked traditional meat products originating from not tested swine, often purchased at the illegal market, was the most common cause of outbreaks. Failure of *Trichinella* testing due to the lack of compliance with standard procedures was also documented. A strategy that includes raising awareness of the producers to meat testing through education campaigns followed by more stringent measures in preventing illegal trade of meat products and the permanent control and certification of laboratories that carry out meat testing are needed in order to reduce the incidence of trichinellosis in Vojvodina and in Serbia.

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