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EPIDEMIOLOGICAL CHARACTERISTICS OF HUMAN LISTERIOSIS IN VOJVODINA, SERBIA, IN THE PERIOD 2005-2020

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Abstract

In the Autonomous Province of Vojvodina, listeriosis has been reported to health authorities since 2005. In this study, we retrospectively analysed the data of listeriosis collected from January 1, 2005 to December 31, 2020. Descriptive method was applied. Demographic, chronological and topographical distribution of the disease in Autonomous Province of Vojvodina was reviewed. In the study period, a total of 52 cases of listeriosis were registered. The average annual incidence rate of reported cases was 0.16/100,000. The largest annual number of cases (n = 10) was reported in 2020. The highest incidence rate registered in youngest age group < 1 year was (5.4/100,000). Gender distribution of the infected population showed no difference with respect to number of infected males and females. Listeriosis was registered throughout the year, but most frequently in October (21.15%). Topographical distribution revealed that listeriosis was more frequently reported in South Bačka county (2.4/100,000 inhabitants). The overall case fatality rate of reported *Listeria monocytogenes* infection was 23.08%. The highest case fatality rate was in the age group 19 - 59 years (29.41%), and fatal outcome in males was twofold the fatality rate in females. Our data analysis suggests that our practitioners, gynaecologists,

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paediatricians, oncologists and other clinicians should take listeriosis into consideration much more often in differential diagnosis in order to improve the diagnosis of the disease and further on epidemiological investigation on source of infection.

Key words: Listeriosis, epidemiology, Vojvodina, APV, epidemiological characteristics

EPIDEMIOLOŠKE KARAKTERISTIKE LISTERIOZE KOD LJUDI U VOJVODINI, SRBIJA, U PERIODU 2005-2020.

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Kratak sadržaj

U Autonomnoj Pokrajini Vojvodini listerioza se prijavljuje nadležnim zdravstvenim organima od 2005. godine. U ovoj studiji smo retrospektivno analizirali podatke o listeriozi prikupljene od 1. januara 2005. do 31. decembra 2020. godine. Primijenjena je deskriptivna metoda. Prikazana je demografska, hronološka i topografska distribucija bolesti u Autonomnoj Pokrajini Vojvodini. U periodu istraživanja registrovana su ukupno 52 slučaja listerioze. Prosečna godišnja stopa incidencije prijavljenih slučajeva ove bolesti bila je 0,16/100.000. Najveći godišnji broj slučajeva (10) prijavljen je 2020. godine. Najveća stopa incidencije registrovana je u najmlađoj starosnoj grupi < 1 godine (5,40/100.000). Polna distribucija obolelih ne pokazuje značajnu razliku između broja obolelih muškaraca i obolelih žena. Listerioza se registrovala tokom svih meseci u godini, ali najčešće u oktobru (21,15%). Topografska distribucija pokazuje da je listerioza češće prijavljivana u Južnobačkom okrugu (2,4/100.000 stanovnika). Letalitet kod registrovanih slučajeva inficiranih bakterijom *Listeria monocitogenes* iznosio je 23,08%. Najveći letalitet bio je u starosnoj grupi 19-59 godina (29,41%). Smrtni ishod je bio dvostruko češći kod muškaraca nego kod žena. Naši

rezultati ukazuju na to da bi ginekolozi, pedijatri, onkolozi i drugi lekari u diferencijalnoj dijagnozi trebalo mnogo češće da uzimaju u obzir listeriozu, kako bi se unapredilo otkrivanje ove bolesti i dalje epidemiološko ispitivanje izvora infekcije.

Ključne reči: listerioza, epidemiologija, Vojvodina, APV, epidemiološke karakteristike

INTRODUCTION

Listeriosis is an infectious disease from a group of zoonoses caused by the bacteria *Listeria monocytogenes*. Although 14 serotypes of these Gram-positive bacteria have been detected so far, only four have been characterized as pathogenic (1/2a, 1/2b, 1/2c and 4b) (Muchaamba et al., 2022). *Listeria monocytogenes* is ubiquitous in nature and can be found in surface waters, soil, vegetation, fodder, silage, sewage and in the digestive tract of many mammals (Bubonja et al., 2007; Mathews, 2008; Hernandez-Milian and Payeras-Cifre, 2014). Animals are mainly asymptomatic carriers of *Listeria monocytogenes*, who shed the bacteria through their faeces and thus can contaminate food sources for other animals and humans (WHO, 2018; Ryser and Marth, 2007). That is why the disease is classified as foodborne.

The main route of transmission of the organism to humans is alimentary, i.e., by consuming contaminated food such as insufficiently pasteurized milk, soft cheeses, uncooked meat delicacies, raw vegetables, frozen foods, raw fish, etc. (Hernandez-Milian and Payeras-Cifre, 2014; Štivić and Tomić Paradžik, 2018; Kiš et al., 2019). *Listeria* can also be transmitted to humans through direct contact with infected animal tissues, especially during lambing or calving (Ryser and Marth, 2007). In addition, transplacental transmission of *Listeria monocytogenes* and transmission during passage of the newborn through the birth canal are important because it can lead to miscarriage, premature birth, neonatal infection and neonatal death (Jackson et al., 2010).

Incubation period lasts from one to 90 days, mostly about three weeks (McLauchlin et al., 2020; Dimitrijević et al., 2008). Two clinical forms of listeriosis can be distinguished: non-invasive and invasive (WHO, 2018). Non-invasive listeriosis is also called febrile listerial gastroenteritis, which is manifested by diarrhoea and flu-like symptoms, and rarely can be registered in this form. Invasive form of the disease is severe condition with a clinical presentation of septicaemia, meningitis or encephalitis. This form of listeriosis is accompanied by high case fatality rate (CFR) of as much as 20 - 30% (WHO, 2018). Pregnant women, neonates, adults over the age of 65 and persons with a compromised

immune system are at risk of developing severe, invasive forms of the disease (Doganay, 2003). Listeriosis is mostly reported sporadically, while outbreaks occur occasionally. The incidence rate across the world ranges from 0.1 to 11.3 cases per 100,000 inhabitants per year (Jeffs et al., 2020). This disease was the fifth most frequently reported zoonosis in the European Union (EU) in 2020, with 1,876 confirmed cases, giving an incidence rate of 0.42 per 100,000 European inhabitants (ECDC, 2020a).

In our province, listeriosis has been reported to health authorities since 2005. The Register of reports of diseases, including listeriosis, is settled at the Institute of Public Health of Vojvodina (IPHV), and it is the only official source of data and evidence on human listeriosis in Autonomous Province of Vojvodina (APV).

APV is located in the north of the Republic of Serbia. It covers an area of 21,506 square kilometres with the population of almost two million inhabitants (RZS, 2014). Vojvodina is territorially divided into seven administrative counties and 45 municipalities.

The aim of this paper was to describe the epidemiological characteristics of listeriosis in APV, Serbia, in the period from 2005 to 2020, for the first time since the disease data have been collected.

MATERIAL AND METHODS

Data collection

The study extended over a sixteen-year period, from January 1, 2005 to December 31, 2020. The collected data of listeriosis were retrospectively analysed using a descriptive method. The data sources included individual reports of infectious diseases cases, epidemiological surveys, and monthly and annual reports. Demographic, chronological and topographical distribution as well as the clinical forms of the listeriosis in APV were reviewed.

Laboratory procedures

Listeria monocytogenes was isolated from blood and cerebrospinal fluid of the patients, or in some occasions from the placenta, amniotic fluid and uterus swab. Classical cultivation of the causative agent on standard media was performed, and the CAMP-test (named for the original authors: Christie, Atkins, and Munch-Petersen) with *Rhodococcus equi* and *Staphylococcus aureus* strains were used to distinguish haemolytic *Listeria* species.

Statistical methods

Data are presented as mean value with the standard deviation (SD), median with interquartile range (IQR) and minimum and maximum values across the groups. The normality assumption was tested using Shapiro-Wilk test with the skewed statistics. To examine the distribution of listeriosis incidence rates throughout the analysed 16-year period between different age groups and county of residence, we used Mann-Whitney U test and Kruskal-Wallis H test with the Dunn's pairwise post hoc comparison using Bonferroni adjustment. All statistical analyses were performed using Stata v.16 (StataCorp LLC. 2019). Results were considered statistically significant when the p-value was < 0.05 .

RESULTS

In the period from 2005 to 2020, a total of 52 cases of listeriosis were registered in APV. Of the total 52 cases included in the study, 12 deaths were registered. The largest annual number of cases ($n = 10$) was reported at the end of the study period, in 2020. The cases of listeriosis in the observed period were reported sporadically and mainly in the form of the severe clinical presentations, with the diagnoses of *listeriosis septica* (40%), *meningitis et meningoencephalitis listerialis* (35%) and *listeriosis non specificata* (25%).

The average annual incidence rate ranged from 0.05/100,000 in 2006 to 0.52/100,000 in 2020 showing an apparent increasing trend during the study period (Figure 1).

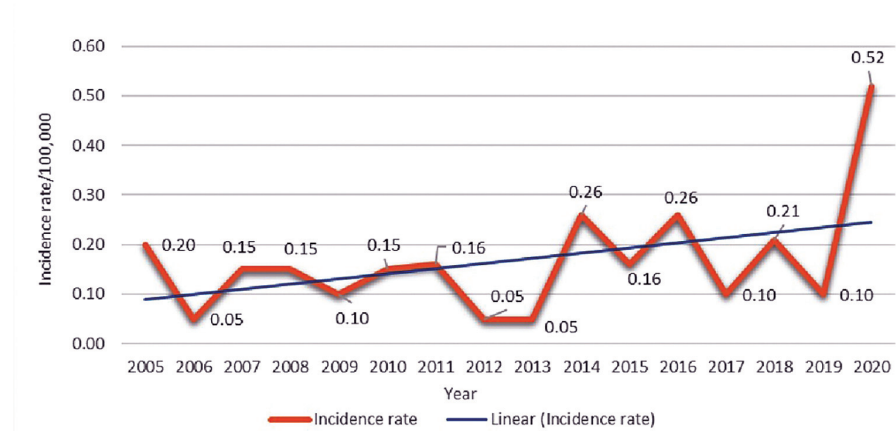


Figure 1. Incidence rate of human listeriosis in APV, Serbia, in the period 2005-2020

The highest age-specific incidence rate was recorded in youngest age group < 1 year (5.40/100,000) (Table 1). Gender distribution of the infected individuals showed no difference between males and females (M : F = 1.08 : 1). The gender-specific incidence rate peaks in age group < 1 among males (6.27/100,000) and the same age group among females (4.44/100,000).

Table 1. Mean incidence rates of listeriosis by age groups and gender in APV, Serbia, in the period 2005-2020

Age group	No of cases		Σ	incidence rate/100000		Σ
< 1	male	9	15	male	6.27	5.40
	female	6		female	4.44	
1-18	male	0	1	male	0	0.02
	female	1		female	0.14	
19-59	male	10	17	male	0.47	0.10
	female	7		female	0.32	
60+	male	8	19	male	0.25	0.25
	female	11		female	0.27	
Total	male	27	52	male	0.17	0.16
	female	25		female	0.15	

The difference between incidence rates of listeriosis among the four age groups (< 1, 1 - 18, 19 - 59 and 60 +) was statistically significant, ($\chi^2 = 18.06$; $p < 0.01$) (Table 2). Multiple comparisons showed that there was a statistically significant difference between age groups < 1 and 1 - 18 ($p < 0.01$) and groups 1-18 and 60 + ($p < 0.01$).

Table 2. Multiple comparisons of incidence rates of listeriosis by age-groups, APV, Serbia, in the period 2005-2020.

Age group (years)	mean incidence	SD	median	IQR		min	max	χ^2	P-value [†]
<1	5.40	7.15	5.70	0	5.80	0	28.90		
1-18	0.02	0.08	0	0	0	0	0.30		
19-59	0.11	0.12	0.10	0	0.15	0	0.40		
60+	0.25	0.26	0.20	0	0.40	0	0.90		

SD= standard deviation; IQR = interquartile range

Indicators of significance between groups using Kruskal-Wallis H test. Dunn's pairwise post hoc comparison was conducted with Bonferroni adjustment.

In APV, listeriosis has been registered throughout the year, but the largest number of cases was reported in October, followed by May, August and September (Figure 2).

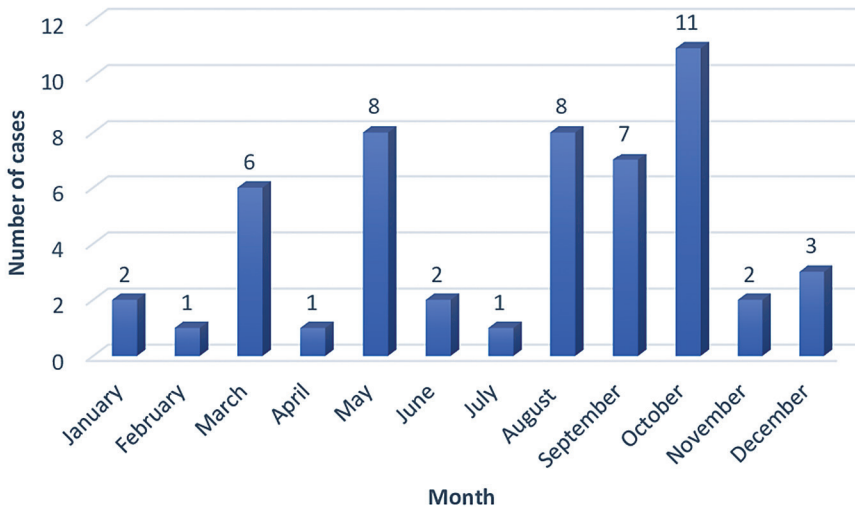


Figure 2. Seasonal distribution of human listeriosis in APV, Serbia, in the period 2005-2020

Topographical distribution revealed that listeriosis was more frequently reported in South Bačka county (incidence rate 2.4/100,000 inhabitants) (Figure 3). Although the incidence rate of listeriosis was higher in South Bačka county than in all other counties there was no statistically significant difference between the counties ($p = 0.55$).

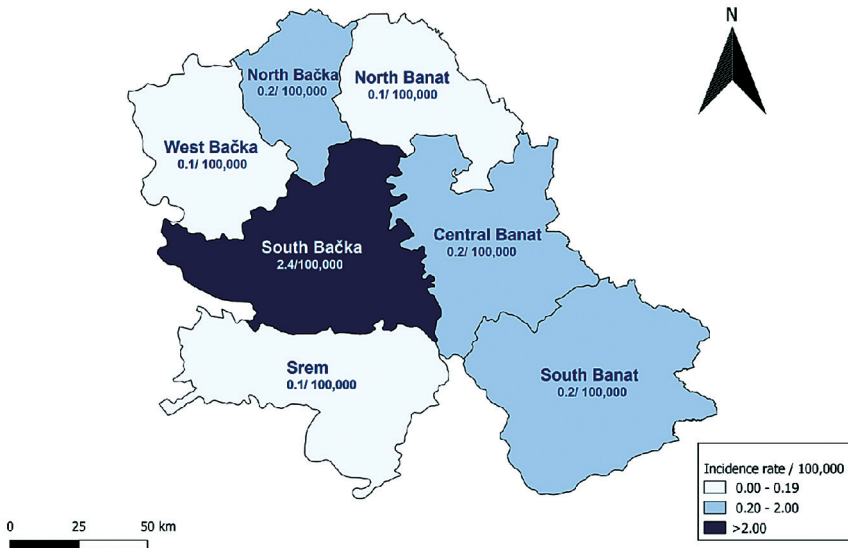


Figure 3. Distribution of human listeriosis in APV, Serbia, by counties in the period 2005-2020

The overall case fatality rate of reported *Listeria monocytogenes* infection was 23.08%. The highest CFR was in age group 19 - 59 (29.41%), and fatal outcome was twice as frequent in males as in females (M : F = 2 : 1).

Table 3. Case fatality rates of listeriosis by age groups and gender, APV, Serbia, in the period 2005-2020

Age group	No of deaths	Σ	Case fatality rate (%)	Σ
< 1	male 0	2	male 0	13.33
	female 2		female 33.33	
1-18	male 0	0	male 0	0
	female 0		female 0	
19-59	male 5	5	male 50	29.41
	female 0		female 0	
60+	male 3	5	male 37.50	26.31
	female 2		female 18.18	
Total	male 8	12	male 29.63	23.08
	female 4		female 16.00	

DISCUSSION

Listeriosis occurs worldwide. It could be a very serious infection with severe clinical symptoms in certain vulnerable groups and with a high case-fatality rate. The highest numbers of cases worldwide have been reported in high-income countries, but the highest burden of the disease is estimated to be in some Latin American countries (Noordhout et al., 2014). To the best of our knowledge, this is the first study conducted in Serbia that summarizes the chronological, topographical and demographic characteristics of human listeriosis in APV.

Our results revealed that the average annual incidence rate of reported invasive cases was 0.16/100,000 inhabitants in APV, which is in accordance with the incidence of the entire territory of Serbia, but lower than the average incidence (0.43/100,000 inhabitants) reported in the EU between 2011 and 2020 (IPHS, 2020; ECDC Atlas, 2020). In the EU, over a seven-year period from 2008 to 2015, the notification rate of invasive cases of listeriosis increased from 0.30 to 0.46 cases per 100,000 inhabitants (EFSA, 2018).

Furthermore, the average incidence recorded in our study is almost two times lower than the annual incidence in the United States (0.28/100,000) between 2008 and 2016 among the general population excluding pregnant women, and four times lower than the average incidence in Germany in the period 2010-2019 (0.69/100,000) (Pohl et al., 2019; Wilking et al., 2021). Due to the high proportion of mild and asymptomatic forms of the disease and their insufficient recognition, the incidence of listeriosis is considered to be significantly higher in APV.

In 2020, an incidence rate of listeriosis in APV reached the highest value of 0.52/100,000. A total of 10 cases were reported during that year, and all of them were investigated; however, due to a long period of incubation and poor anamnestic data the source of the infection and epidemiological association of cases could not be determined, thus, the cause of this increase of the incidence rate remains unclear. It is likely that during the COVID-19 pandemic the clinicians were paying more attention to infectious diseases, including listeriosis. Even though the notification rate of listeriosis in 2020 has improved in the APV, it is still lower than that reported by some European countries. In the same year, the highest incidence rates in the EU were reported in Finland, Slovenia, Malta and Sweden with 1.70, 1.20, 0.97 and 0.85 cases per 100,000 inhabitants, respectively (ECDC, 2020a). Finland reported the highest number of cases of listeriosis per year in Europe, most probably due to the high consumption of cold-smoked and dried salmon (Pasonena et al., 2019).

All cases of listeriosis reported in Vojvodina during the 16-year study period occurred sporadically, with no epidemiological association established. In the EU, from 2010 to 2016, 63 outbreaks were reported (ECDC, 2018). There is an example of the outbreak in South Africa, showing that it could be very massive and fatal with 1050 persons infected and 216 deaths (CIPH, 2020).

We further established that the highest age-specific incidence was registered at the age of < 1 year, while according to the ECDC data the largest portion of human listeriosis cases was observed in the age group over 64 years (ECDC, 2020a). A study conducted in Germany in the period 2010-2019 revealed that the highest incidence rate was recorded among adults ≥ 85 years (Wilking et al., 2021). Our findings showed that listeriosis occurred almost equally in males and females, while in Austria, in the period from 1997 to 2007, the male to female ratio of listeriosis was 1.23 : 1 (Kasper et al., 2009). A similar sex ratio was registered in the EU (ECDC, 2020b).

Our results revealed that the diagnosis of septic listeriosis was dominant in the age group < 1 (86.67%), and the diagnosis of listeriosis meningitis prevailed in the age group 60 + (73.68%). In a study conducted in Turkey between 1987 and 2001, sepsis was diagnosed in 21 - 43% of cases, and 56% of central nervous system infections (Doganay, 2003).

Out of 25 female cases with the notification of *Listeria monocytogenes* in this study, seven were pregnant, although, six were detected during postpartum period. In the study conducted in the United States covering the period 2004-2007, about 16% of reported listeriosis cases were in pregnant woman (Jackson et al., 2010). These data are not surprising considering that pregnant women have greater risk of *L. monocytogenes* infection due to the immunosuppression during pregnancy. Also, infection with this bacterium can cause premature births, miscarriages or stillbirths, thus, pregnant women and women in the postpartum period are more often laboratory tested for listeriosis (Jeffs et al., 2020).

In APV, listeriosis was registered throughout the year, but the largest number of cases was reported in May, August, September and especially in October. A study conducted in Israel from 1997 to 2007 reported almost the same seasonal distribution, and the largest proportion of cases (64.2%) was reported from May to October (Vasilev et al., 2009). Almost the same seasonal summer peak was reported by ECDC (ECDC, 2020b). It is assumed that more frequent occurrence of other gastrointestinal infections during the warmer months is related to the increase in the number of listeriosis patients, as the causative agents of these infections may cause immunosuppression and facilitate the entry and spread of *L. monocytogenes* in the gastrointestinal tract (Schwartz et al., 1989; Vasilev et al., 2009).

The largest number of registered cases in the South Bačka county can be explained by the fact that the main administrative centre and the largest city in APV (Novi Sad) is situated on the territory of this county, where diagnostic possibilities, disease reporting and service networks are more developed.

Overall case fatality rate of 23.1% recorded during our study period, as well as 33.3% in 2019 in the entire Serbia and 13.0% in 2020 in EU, make listeriosis one of the most serious human foodborne diseases (IPHS, 2020; ECDC, 2020a).

CONCLUSION

Listeriosis is an important public health problem in Vojvodina. Control measures of listeriosis rely on the safe production, distribution and preparation of food of animal origin, as well as on health education in the general population, primarily in vulnerable groups.

The analysis of our data shows that our practitioners, gynaecologists, paediatricians, oncologists and other clinicians should take listeriosis into consideration much more often in differential diagnosis in order to improve the diagnosis of the disease and further on epidemiological investigation on source of infection. Education and raising awareness about this disease at all levels of health care are essential for recognition and better notification of listeriosis. Timely exchange of information and institutional collaboration between public health and veterinary services is also very important to improve listeriosis surveillance.

Author's Contribution:

NJ, TP and GD made contributions to the basic idea, conception and design of the article; NJ and SR were involved in the data collection; NJ drafted the manuscript; GD coordinated the work and revised the manuscript and participated in the final draft of the manuscript; VV performed statistical analysis; NJ, TP, VV, SR and GD contributed in data analysis and interpretation of results. All authors have read and approved the final manuscript.

Competing interest

The authors declare that they have no competing interest.

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