

PREVALENCE OF GIARDIASIS IN ASYMPTOMATIC INDIVIDUALS IN THE TERRITORY OF THE CITY OF NIŠ

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Giardiasis/Lambliasis is a disease caused by the presence of *Giardia lamblia* (*G. lamblia*) in the upper portions of the human digestive tract (DT). Our aim in this paper was to determine the prevalence of giardiasis/lambliasis in asymptomatic individuals subject to sanitary supervision in the territory of the city of Niš.

We performed a retrospective analysis of stool sample test results of individuals without any symptoms of a digestive tract infection (January 2004 - December 2014) using conventional microscopy (CVM). The results were subsequently statistically analyzed.

The study group (341650) consisted of people subject to sanitary supervision. By way of CVM, *G. lamblia* was identified in 574 individuals (1.68 per 1000 inhabitants), with mean age 33.76 ± 12.93 years, equally in both sexes. In the analyzed period there was a statistically significant reduction in the prevalence of giardiasis ($p < 0.001$). The highest prevalence was found in 2005 (4.90) and the lowest in 2014 (0.57 per 1000 inhabitants). Giardiasis was most prevalent in retail workers (27.20%), workers in food preparation and food industry (18.10%) and caterers (12.70%). With regards to seasonal prevalence, the highest prevalence was in the summer (31.0%) and the lowest in the autumn (18.50%).

In the Niš region, the prevalence of asymptomatic giardiasis is significantly reduced in individuals that are subject to sanitary controls for systematic monitoring and implementation of measures of prevention and control of giardiasis. In patients with digestive complaints for the infective etiology of the disease to be excluded, it is necessary to perform parasitic examinations of the samples in order to confirm or exclude the infection with this protozoan. *Acta Medica Medianae* 2017;56(3):48-54.

Key words: giardiasis/lambliasis, prevalence, sanitary supervision

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Introduction

The first report of the protozoan *Giardia lamblia* (*G. lamblia*) 325 years ago was presented by Leeuwenhoek after examining his own watery stools under microscope (1). Although it is one of the oldest eukaryotes, *G. lamblia* is still an interesting and puzzling organism for humans. Numerous scientific projects promise significant advances yet to be made regarding the knowledge about this unicellular flagellate.

Giardiasis/lambliasis is a disease caused by the presence of *G. lamblia* in the upper portions of the human digestive tract (DT). Infectious *G. lamblia* cysts can enter the organism via conta-

minated water, food, or by direct fecooral transmission. A single cyst is all that takes for this infection to occur, although the studies enrolling volunteering subjects have shown that usually 10-25 cysts are required (2).

The period of incubation is 3-25 days (7-10 days, on the average), after which the disease symptoms develop. The symptoms of giardiasis are very diverse. Giardiasis should be suspected if symptoms last for a week or longer, two weeks at the most. An episode of diarrhea lasting less than a week, untreated, is probably not giardiasis. The disease can persist for several months if left untreated, and can be described as a continual worsening of diarrheal symptoms (3, 4). In chronically affected individuals, malabsorption of fats, lactose, and vitamin A and B has been reported, and in children a slower weight gain and growth retardation have been observed (2).

However, there are patients without any of the above symptoms. Throughout the world, most of the patients infected with the protozoan are in fact asymptomatic (2). It is still not known whether these infections are the result of infection with a non-pathogenic variety of the organism, or

their immune systems are able to maintain the number of parasites at a low level (5).

Conventional microscopy (CVM) of three stool samples, with or without concentration technique, is still considered a reference method (a „gold standard“) for the diagnosis of DT infection with *G. lamblia* parasite. Near the end of the last century, the introduction of new diagnostic immunoenzyme (EIA) tests in parasitology laboratories is a turning point in the diagnosis of giardiasis. Demonstration of antigen presence in the feces utilizing immunoabsorption enzyme assays, non-enzymatic immuno-tests, and/or fluorescein-labelled monoclonal antibodies can be superior diagnostic methods (6-12). It is especially important in disease course evaluation or in the screening for *G. lamblia* infection, particularly in epidemiological research. In patients in whom parasitological diagnosis has not been made, endoscopy with duodenal fluid sampling is a sound option, as well as the biopsy of duodenal or gastric mucosa (4, 13).

Cultivation and sensitive tests using the molecular methods, such as DNA probe and PCR, are available only in research centers and are not routinely employed. DNA probes are usually used to prove the presence of parasites in water samples, while PCR is most commonly used in molecular epidemiology and studies of the zoonotic and pathogenic potential of *G. lamblia* (14-17).

After the treatment of individuals with giardiasis and absence from work due to illness, a parasitology control of the samples of three consecutive stools to detect the presence of *G. lamblia* cysts, or demonstration of *G. lamblia* antigen in the stool using immunodiagnostic procedures are mandatory. After negative findings have been obtained, the cured patient can return to his working environment (2).

Systematic research of giardiasis in the Nis region was carried out ten years ago (2). Further monitoring of this parasitosis is necessary in order to perceive fully the epidemiological situation in our region.

Aim

Our aim in the study was to establish the prevalence of *G. lamblia* protozoan in the stool samples from healthy, asymptomatic persons on sanitary surveillance/supervision.

Material and methods

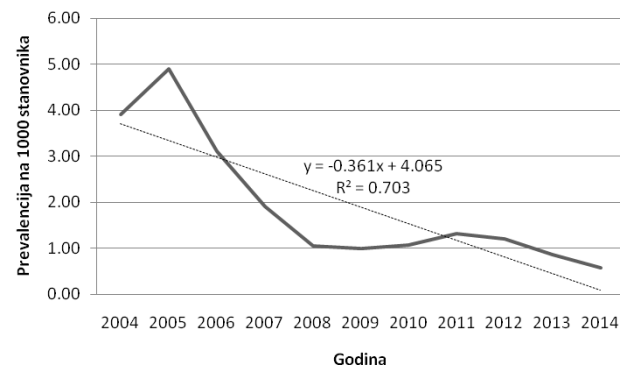
Groups of examinees. The study was done in the Parasitology Laboratory, Center for Microbiology, Public Health Institute in Niš, in the period from January 2004 to December 2014. The study involved healthy individuals (341.650) from the Niš region, without any symptoms of digestive tract infection, on mandatory occupational sanitary supervision (the so called „sanitary examinations“).

Sample processing. Parasitological examination of fresh stool samples, without conservation, involved microscopy of one stool sample using CVM. A microscopic examination of the stool for *G. lamblia* involved the use of a directly stained native preparation. A direct native stained preparation was made of fresh stool samples. A drop of Lugol's iodine (the solution of potassium iodide which enables visualization of *G. lamblia* cyst structure) was placed onto the microscopic slide in question with a small amount of feces (around 2mg) in it and was well emulgated with a wooden stick. A covering glass slide was placed onto the slide, sufficiently thin (22-50 mm), and then visualized using a light microscope and a small (x10) and greater objective magnification (x40) in order to detect *G. lamblia* cysts. Lugol's iodine stained the cyst nuclei yellowish-brown, and glycogen within the vacuoles reddish-brown (2, 12, 18, 19). The preparations were examined under a microscope within the period of 15 minutes after the preparation. The investigation was considered positive if a cyst (cysts) of *G. Lamblia* was detected.

Statistical analysis. The results were systematized and processed using the statistical method of descriptive and quantitative analysis of the SPSS [Statistical Package for Social Sciences (SPSS) 14.0 for Windows 2003]. Comparisons of arithmetic means of a pair of samples was done using the t-test for independent samples. Comparison of the frequency of attributive characteristics was performed using the χ^2 -test or Fisher's exact probability test when the obtained frequency was below 5. The values of $p < 0.05$ were considered statistically significant.

Results

In the studied population (341.650) in the period from January 2004 to December 2014, there were 574 positive findings, representing the prevalence of asymptomatic giardiasis of 1,68 per 1000 people. The highest value of prevalence was obtained for 2005 in the examined group (4.90), and the lowest for 2014 (0.57 per 1000 people). It



Graph 1. Prevalence of giardiasis/lambliasis in the period 2004-2014

Table 1.: Prevalence of giardiasis/lambliasis per 1000 inhabitants in the period 2004-2014

Year	Nº of examinations	Nº of positives	Prevalence per 1000 inhabitants
2004	14833	58	3.91
2005	23875	117	4.90
2006	31230	97	3.11
2007	33184	63	1.90
2008	33563	35	1.04
2009	33482	33	0.99
2010	33676	36	1.07
2011	34028	45	1.32
2012	33633	40	1.19
2013	35190	30	0.85
2014	34956	20	0.57
Total	341650	574	1.68

was established that the reduction of prevalence of asymptomatic giardiasis in the studied period was statistically significant ($p < 0.001$) (Table 1 and Graph 1).

The number of positive findings was distributed equally across genders: there were 278 male (48.40%) and 296 female examinees (51.60%) with positive findings. The examinees had 33.76 ± 12.93 years on the average and there were no statistically significant differences in age distribution across genders ($p = 0.245$) (Table 2).

Regarding the occupation/profession of the examinees, positive findings were encountered mostly in shopworkers (27.20%), in people employed in food preparation and in food industry (18.10%), and in hospitality industry (12.70%) (Table 2).

In predominantly male occupations such as drivers of motor vehicles and in manual workers, the prevalence of positive findings was statistically significantly higher compared to female examinees ($p < 0.001$). In male workers in the hospitality industry, positive findings were statistically significantly more common than in women ($p = 0.021$). People working in the health care sector, administration, and education sectors, had statistically significantly more common positive findings of *G. lamblia* if they were of female gender ($p < 0.001$, i.e. $p = 0.002$) (Table 2).

The number of positive findings was much higher in asymptomatic individuals living in urban areas (322/56.10 per 1000 inhabitants) than in those living in rural environments, while the number of women living in urban areas with asymptomatic giardiasis (181/61.10 per 1000 inhabitants) was statistically significantly higher compared to that in men ($p = 0.015$) (Table 2).

Throughout the year, positive findings of *G. lamblia* were mostly encountered in the summer (31.00%), while these were rarest in the autumn (18.50%). It was established that positive findings were statistically significantly more common in

Table 2.: Positive findings of *G. lamblia* by age, gender, occupation, and place of residence

Parameter	Category	Total	Gender		p
			Male	Female	
Age			33.11±13.67	34.37±12.18	0.245
Occupation	Children	15 (2.60)	8 (2.90)	7 (2.40)	0.700
	Pupils/students	74 (12.90)	42 (15.10)	32 (10.80)	0.125
	Drivers of motorized vehicles	22 (3.80)	22 (7.90)	0	<0.001
	Manual workers	82 (14.30)	56 (20.10)	26 (8.80)	<0.001
	Hospitality industry workers	73 (12.70)	45 (16.20)	28 (9.50)	0.021
	Food preparation and food industry workers	104 (18.10)	52 (16.20)	52 (17.60)	0.723
	Health professionals	19 (3.30)	1 (0.4)	18 (6.10)	<0.001
	Workers in administration and education	20 (3.50)	3 (1.10)	17 (5.70)	0.002
	Shopworkers	156 (27.20)	46 (16.50)	110 (37.20)	<0.001
	Other	9 (1.60)	3 (1.10)	6 (2.00)	0.361
Residence	Rural	252 (43.90)	137 (49.30)	115 (38.90)	0.015
	Urban	322 (56.10)	141 (50.70)	181 (61.10)	

Table 3.: Seasonal character of I giardiasis/lambliasis related to gender

Parameter	Category	Total	Gender		p
			Male	Female	
Season	Winter	165 (28.70)	86 (30.90)	79 (26.70)	0.302
	Spring	125 (21.80)	46 (16.50)	79 (26.70)	0.004
	Summer	178 (31.00)	94 (33.80)	84 (28.40)	0.188
	Autumn	106 (18.50)	52 (18.70)	54 (18.20)	0.97

women in the spring, compared to men ($p=0.004$) (Table 3).

Discussion

In numerous papers, the data can be found about the general prevalence of giardiasis worldwide and about morbidity in some of the countries or parts of the countries, offering valuable information that can be used in the assessment of exposure and risk of the infection in certain population groups (2, 19). *G. lamblia* is thought of as the most common helminthiasis of the digestive tract, in both technologically advanced and in less developed communities (20).

The Center for disease control and prevention (CDC) has presented the information that in the USA almost one case of giardiasis is registered yearly per each 100 individuals. In 2009-2010, the total number of cases reported in the USA rose by 1.9%, but the infection rate remained stable (7.3-7.6 cases per 100.000 people) (21). Moreover, 200 million people in Asia, Africa, and Latin America have got giardiasis, with around 500.000 new cases of the infection reported per year. The prevalence of giardiasis in individual countries of the mentioned large geographical areas is as follows: 10% in Panama; 9% in Venezuela; 4-11% in Brazil; 4-15% in Egypt; and 10% in China (2, 21). In northern Europe, the principal threat constitute in fact the imported cases of giardiasis, while the prevalence of giardiasis in southern parts of Europe is nevertheless high, especially in Spain (2, 21). As for the endemicity of giardiasis, the World Health Organization (WHO) has set the norms for the European countries. Taking them into consideration, the level of infection above 10% is denoted as hyperendemicity, the level around 1% is endemicity, and anything below 1% is denoted as sporadic disease (2, 22).

In the territory of ex-Yugoslavia, giardiasis was analyzed by a group of WHO experts at the end of the 1970s. Based on their results, immediately after the Second World War, Yugoslavia was categorized among the countries with the percentage of *G. lamblia* infection ranging from 2.5% to 35.3% (23). The first papers about giardiasis in the territory of ex-Yugoslavia appeared between the two world wars. In the 50 years to follow, a large number of published papers presented the results of investigation of giardiasis in all ex-Yugoslavia regions. Simić et al. established the presence of the protozoan in Sumadia with the prevalence of 3.2% to 18.5%. In the 1980s, Antonović systematically studied the spread of giardiasis in the territory of Serbia without provinces in preschool and school children. He also examined the prevalence of giardiasis in adults in two localities of the Zaječar region and established the percentage of 1.79% (23).

In the 1990s, Nikolić performed a study to examine the prevalence of bowel parasitic infections in children aged 7-11 years in 16 areas of the central Serbia, encompassing 91 populated localities. She discovered 9 types of parasites, and

in 6.8% of the examined subjects overall she detected *G. lamblia*, with a significant difference between the minimal and maximal prevalence of giardiasis, from 3.2% to 14.2% related to the studied region and locality (2).

The surveillance of prevalence of asymptomatic giardiasis in workers in food industry in the territory of Serbia was performed as early as the beginning of the 1960s. A very high prevalence of 9% of cyst carrier status was reported. Owing to the undertaken epidemiological measures, i.e. mandatory health supervision examinations, the prevalence of asymptomatic giardiasis in the general population was reduced near the end of the 1970s to only 0.8% (23). In the same period, there were 2.56%-2.60% of cyst carriers in the Nišava district among the people undergoing an increased health surveillance and control program (23), and near the end of the 1980s the proportion was reduced to 0.8% (25).

A systemic investigation of giardiasis in the city of Niš territory was performed in the period from 2003 to 2006. This large study involved examination of the presence of *G. lamblia* cysts in the digestive tract of individuals with symptomatic giardiasis, asymptomatic individuals with an increased risk of infection, and asymptomatic individuals on sanitary supervision, using CVM and direct immunoenzyme assay to establish the presence of *G. lamblia* antigen in the feces. The prevalence of asymptomatic giardiasis in high risk individuals was rather high, 6%, in psychiatric patients, 5.85% in refugees, expelled, and displaced (RED) persons, to as high as 12% in pediatric RED persons. In those on sanitary supervision, the prevalence of giardiasis of 0.41% (1/241) was established using the CVM of a single stool sample (2).

The results of our study demonstrated that the trend of reduction of prevalence of asymptomatic giardiasis continued. During our eleven years long monitoring of prevalence of asymptomatic giardiasis in individuals on sanitary surveillance, there were 574 positive findings, reflecting the prevalence of 1.68 per 1000 inhabitants. The highest prevalence was reported for 2005 (4.90 per 1000 inhabitants), with a subsequent reduction during the studied period ($p < 0.001$). In 2014, the lowest prevalence of asymptomatic giardiasis was found in the city of Niš territory (0.57 per 1000 inhabitants). This constant reduction of prevalence of giardiasis in healthy persons on sanitary surveillance/ supervision can be attributed to the Law on Protection of Population from Contagious Diseases (26) that regulates the measures of protection of the population from contagious diseases, their mode of implementation and provision of means for their implementation, implementation control, and other issues relevant in the protection of population from contagious diseases. The results similar to our own have been obtained in the USA, where the reporting of giardiasis has been compulsory since 2002. The compulsory reporting and surveillance of *G. lamblia* in the USA has produced favorable epidemiological effects, since

there has not been any dramatic increase of the prevalence of giardiasis in this country (21).

Based on the information gained via giardiasis surveillance in the USA, a bimodal age-related distribution of the parasitosis has been noticed. Most of the infected with *G. lamblia* are children aged 1-9 years, followed by adults aged 35-49 years. A higher prevalence of giardiasis in children could perhaps be explained by the use of recreational objects (pools, gyms, etc.), poor sanitary and hygienic conditions, absence of adequate hygiene skills, close contact with potentially infected children and household members, and direct contacts with animals (20, 26). As for the gender, men were more frequently infected (56.2% in 2009 and 56.7% in 2010) in all age groups (with the most conspicuous situation in men aged 35-54 years) (21).

Although men were more frequently infected with *G. lamblia* protozoan, the number of positive findings was approximately equal for both genders in our study: 278 male (48.40%) and 296 female examinees (51.60%), aged 33.76 ± 12.93 years on the average ($p=0.245$). During the study, the percentage of positive findings in children was 2.60% and in school children (below 19 years of age) 12.90%. A lower number of positive findings in the population of children and younger persons in this study was something to be expected, since a sanitary surveillance/supervision program most commonly involved the working age population, so that bimodal age distribution of giardiasis was not observed here.

Significant variations of giardiasis prevalence figures were found related to the occupation of the examinees as well. Positive findings were encountered mostly in shopworkers (27.20%), in those engaged in food preparation, in food industry workers (18.10%), and in hospitality industry (12.70%). These individuals come into contact with large numbers of different people, and without adequate hygiene (hand washing primarily) infection is more likely to occur. Moreover, if there are no adequate personal hygiene habits, the chances for the infection to be transmitted are greater not only via personal contacts, but also via food (21, 27).

As expected, in predominantly male occupations (drivers of motor vehicles and manual workers) the frequency of positive findings of the studied protozoan was statistically significantly higher in men compared to women ($p < 0.001$). Further, in hospitality industry workers, positive findings were statistically significantly more common in men than in women ($p=0.021$). In traditionally female occupations (health care, education), *G. lamblia* was statistically significantly more frequently found in female than in male examinees ($p < 0.001$, i.e. $p=0.002$).

A much higher number of positive findings was found in urban dwellers (322/56.10 per 1000 inhabitants) than in rural ones, similar to the statistically significantly higher number of positive findings in urban women (181/61.10) than in men from urban/rural areas ($p=0.015$). In view of the continuing rural-to-urban migrations, giardiasis is more common in urban than in rural areas. A higher number of positive findings in urban women was probably caused by the occupation of the women subject to health surveillance/supervision programs.

As for the seasonal occurrence of giardiasis throughout the year, most positive findings were encountered in the summer (31.00%) and the lowest number in the autumn (18.50%), although women more commonly than men had asymptomatic giardiasis in the spring ($p=0.004$). Population health surveillance in the USA has shown a doubled number of reported cases of giardiasis in August, and the lowest number in December. The number increased in the middle of May, reached its maximum in August, and from September onwards the number of the infected dropped. Increasing numbers of the reported cases of giardiasis during the summer is common with other enteric bacterial or parasitic diseases as well, for instance with cryptosporidiosis (21). The phenomenon can be explained by an increased level of activity in the open during the summer, possibly more common defecation in the open, and by biological characteristics of *G. lamblia* (cyst endurance, low infectious dose, intermittent and long-term cyst disposition) (21, 27).

Conclusion

The results of the epidemiologic research show that the prevalence of asymptomatic giardiasis in the general population in the city of Niš territory is low. A systematic surveillance of giardiasis and institution of prevention and control measures have contributed to a significant reduction of prevalence of this parasitic disease, especially in asymptomatic carriers on mandatory sanitary supervision. A further reduction of prevalence of giardiasis would require continued surveillance of the parasitosis and improvement of health education of the general public, as well as the introduction of up-to-date diagnostic procedures for epidemiological surveillance of giardiasis. On the other hand, in patients with digestive complaints for the infective etiology of the disease to be excluded it is necessary to perform parasitic exams of the samples (stool, duodenal fluid, etc.), and to require other, supplementary non-invasive and invasive diagnostic procedures in order to confirm or exclude the infection with this protozoan.

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Originalni rad

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doi:10.5633/amm.2017.0308**PREVALENCIJA ĐARDIOZE KOD ASIMPTOMATSKIH OSOBA NA PODRUČJU GRADA NIŠA***Nataša Miladinović Tasić¹, Nikola Đorđević², Dragan Zdravković¹, Aleksandar Tasić¹*Institut za javno zdravlje, Niš, Srbija¹
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Đardioza/lambliozna je oboljenje uzrokovano prisustvom protozoe *Giardia lamblia* (*G. lamblia*) u gornjim partijama digestivnog trakta (DT) čoveka. Cilj rada bio je da se utvrdi učestalost đardioze/lamblioze kod asimptomatskih osoba na sanitarnom nadzoru.

Izvršena je retrospektivna analiza rezultata pregleda uzoraka stolice zdravih ljudi bez simptoma DT (januar 2004 – decembar 2014.), korišćenjem konvencionalne mikroskopije (KVM). Dobijeni rezultati su statistički analizirani.

Ispitivanu grupu (341650) činile su zdrave osobe, bez simptoma infekcije, na zakonom propisanom obaveznom vidu zdravstvenog pregleda (sanitarna kontrola). Analizom uzoraka stolice dobijen je pozitivan nalaz *G. lamblia* kod 574 osobe (1,68 na 1000 stanovnika), prosečne starosti $33,76 \pm 12,93$ godiina, podjednako u oba pola. U ispitivanom periodu došlo je do statistički značajnog smanjenja prevalencije đardioze ($p < 0,001$). Najviša prevalencija đardioze utvrđena je 2005. godine (4,90), a najmanja 2014. godine (0,57 na 1000 stanovnika). Najčešće je đardioza bila zastupljena kod radnika u trgovini (27,20%), kod radnika na pripremi hrane, u prehrambenoj industriji (18,10%) i kod ugostitelja (12,70%). U odnosu na sezonsko javljanje, najviše je pozitivnih nalaza u leto (31,0%), a najmanji u jesen (18,50%).

U niškom regionu, prevalencija asimptomatske đardioze je značajno smanjena kod osoba na sanitarnim kontrolama za sistematsko praćenje i sprovođenje mera prevencije i kontrole đardioze. Kod bolesnika sa digestivnim tegobama, posebno kod dece, neophodno je pri isključivanju infektivne etiologije bolesti tražiti i parazitološki pregled uzoraka u cilju potvrde ili isključivanja infekcije ovom protozooom. *Acta Medica Medianae* 2017;56(3):48-54.

Ključne reči: đardiozis/lambliozis, prevalencija, sanitarni nadzor

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