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Adriana Codrean, Dan L. Dumitrascu, Victor Codrean, Delia Mirela Tit,  
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Nistor-Cseppento, Lotfi Aleya, et al.

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1                   **Epidemiology of human giardiasis in Romania: a 14 years survey**

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3   Adriana Codrean<sup>1</sup>, Dan L. Dumitrascu<sup>2</sup>, Victor Codrean<sup>3</sup>, Delia Mirela Tit<sup>4</sup>, Simona Bungau<sup>4</sup>,  
4   Selim Aleya<sup>5</sup>, Marius Rus<sup>6</sup>, Ovidiu Fratila<sup>6</sup>, Delia Carmen Nistor-Cseppento<sup>1</sup>, Lotfi Aleya<sup>7\*</sup>,  
5   Nicoleta Negrut<sup>1</sup>

6

7   <sup>1</sup>Department of Psycho-Neuroscience and Recovery, Faculty of Medicine and Pharmacy,  
8   University of Oradea, Oradea, 410028, Romania; j.adriana9@gmail.com (A.C),  
9   lnm\_n10@yahoo.com (N.N), delia\_cseppento@yahoo.com (D.C.N.-C.)

10   <sup>2</sup>2<sup>nd</sup> Medical Department, “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-  
11   Napoca, Romania; dan\_dumitrascu@yahoo.de;

12   <sup>3</sup>Faculty of Environmental Protection, University of Oradea, Oradea, Romania,  
13   victorcodrean34@yahoo.com;

14   <sup>4</sup>Department of Pharmacy, Faculty of Medicine and Pharmacy, University of Oradea, Oradea  
15   410028, Romania; mirela\_tit@yahoo.com\_(D.M.T.), simonabungau@gmail.com\_(S.B.);

16   <sup>5</sup>Faculty of Medecine, Université de Franche-Comté, Besançon, France;

17   <sup>6</sup>Department of Medical Disciplines, Faculty of Medicine and Pharmacy, University of  
18   Oradea, Oradea 410028, Romania; rusmarius@yahoo.com (M.R.) ovidiufr@yahoo.co.uk  
19   (O.F.)

20   <sup>\*7</sup>Chrono-environnement CNRS 6249, Université de Franche-Comté, Besançon, France;

21

22   \*Corresponding author: lotfi.aleya@univ-fcomte.fr

23   Phone: + 33 (0) 3 81 66 66 66

24   Fax: + 33 (0) 3 81 66 57 97

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## **Abstract**

*Giardia* spp. is the most common intestinal protozoan (causing gastrointestinal illness) and the most frequent cause of parasitic gastroenteritis in humans and animals worldwide. The aim of this study was to highlight new data in a specific area regarding clinical presentation and epidemiological prevalence over a long period of time. Patients (a total number of 54,623 patients) admitted in a tertiary center for infectious diseases serving a county for a period of 14 years were tested for infection with *Giardia*. Positive cases were recorded through analyzing the clinical complaints, the month of incidence and the demographic area from which the patients came from. Longitudinal trends have been evaluated. The incidence of giardiasis among the tested patients was 4.47%. A decreasing trend was observed regarding the annual incidence. Patients between the ages of 15 and 44 presented most commonly giardiasis, especially those from urban areas and women. The most common symptoms are loss of appetite (71.24%) and abdominal pain (69.07%). The highest monthly incidence was quoted in July (10.65%), August (10.49%) and June (10.20%). This epidemiological study allows a better knowledge of the infection with *Giardia* spp. It gives the long-term changes in demographic characteristics of the infected patients in a specific area and the monthly incidence.

**Keywords:** giardiasis; epidemiology; parasitic intestinal diseases; prevalence; digestive symptoms; zoonoses

## 49 1. Introduction

50           Giardiasis is a digestive zoonotic disease caused by the protozoan genus *Giardia* [*G*],  
51 and the most frequent species is *G. duodenalis* (*syn. G. intestinalis, G. lamblia*) (Acha and  
52 Szyfres, 1992). It is considered as the most common intestinal protozoan and the most  
53 frequent cause of parasitic gastroenteritis around the world (Ignatiu et al., 2012), with  
54 approximately  $2.8 \times 10^8$  new cases emerging every year (Lanes and Lloyd, 2002). *G. lamblia* is  
55 a ubiquitous infection and is more frequent in children than adults, especially in areas with a  
56 warm climate (Eissa and Amer, 2012). In Romania, the infection with *G. lamblia*, along with  
57 other intestinal parasitic infections (ascariasis and enterobiasis), is the most frequent  
58 diagnosed intestinal parasitic disease (Neghina et al., 2011).

59           This flagellated protozoan is transmitted either through direct contact, on a fecal-oral  
60 route, or through consumption of water or foods that are contaminated with cysts of *Giardia*  
61 spp. (Ehsan et al., 2015; Hooshyar et al., 2019), the infectious and resistant form of the  
62 parasite in nature. Cysts are extremely resistant to vitreous environmental conditions and able  
63 to keep their ability to infect for months. Therefore, there is a real risk of contamination of  
64 and from the environment with *Giardia* cysts. Water samples (wastewater from any source:  
65 surface water, drinking water, swimming pools) taken anywhere in the world have confirmed  
66 this infestation, with numerous outbreaks of germination in many countries (Sărmășan et al.,  
67 2008). *Giardia* is the second most common cause of gastroenteritis caused by water infested  
68 with protozoan parasites (Boarato-David et al., 2017). The presence of the *Giardia* spp. cysts  
69 in the water is also due to the fact that they are largely spread in the environment and are  
70 generally fairly resistant to environmental factors. In both human and animals, this protozoan  
71 has a great clinical value (Olson, 2004). The main zoonotic-epidemiologic issues that these  
72 parasitic zoonoses raise are those concerning the surveillance, prevention and limitation  
73 (Suteu, 2002).

74 It can manifest in endemic form, especially in tropical and subtropical areas, or in an  
75 epidemic form (that affects the travelers in the endemic areas, as it is believed that 2-3% of  
76 the travelers' diarrheas are caused by *Giardia* spp.). It can also occur in restricted  
77 communities, like families or groups from areas with precarious hygiene, or it can manifest  
78 like a hydric epidemic, after the consumption of contaminated water from uncontrolled  
79 sources. The World Health Organization (WHO) estimates that *Giardia* spp. is one of the  
80 parasites that is most commonly spread in humans and animals, which get infected through  
81 water with a parasitic germ that is rarely lethal (Meyer and Radulescu, 1979; Dubourg et al.,  
82 2018). This constitutes a major concern for public health in both developed and developing  
83 countries (Hoque, 2002). The incidence of the infection with *Giardia* spp. varies greatly  
84 according to geographical regions and in the different regions of the same country. The  
85 prevalence is greatly influenced by the diagnostic methods that are used and by the expertise  
86 of those who diagnose the disease.

87 The clinical presentation of the diseases varies greatly, primarily depending on the  
88 location of the parasite, the individual factors (the immune response) and the parasitic  
89 virulence (Judea-Pusta et al., 2019), the dose of the parasite and the duration of the infection,  
90 which can be symptomatic or asymptomatic, with chronic or acute diarrhea, along with  
91 dehydration, weight loss, abdominal pain, nausea and vomiting (Eckmann, 2003; Hooshyar et  
92 al., 2009). The chronic infection with *G. duodenalis* can lead to chronic gastroenteritis,  
93 malnutrition, persistent dysbiosis, zinc deficiency, vitamin A deficiency, iron deficiency and  
94 growth deficiency (Thomson, 2008; Beatty, 2017; Gabor-Harosa et al., 2016; Choy et al,  
95 2014). The extra-digestive symptoms are also an important part of the clinical panel of  
96 giardiasis, especially the allergic, neuropsychiatric and general ones (Einarsson, 2016;  
97 Hooshyar et al., 2019).

98           The aim of our study was to find new epidemiological data on the infection with  
99 *Giardia* spp. and to look for factors influencing the incidence and clinical presentation.

100

## 101 **2. Materials and Methods**

### 102 **2.1. Protocol**

103           A comprehensive 14 years prospective screening from January 1, 2004 until  
104 December 31, 2017, was undertaken in a single center for infectious diseases, with large  
105 referral area. All patients admitted to this center were tested for *Giardia* spp. infection. All the  
106 subjects were tested, whether symptomatic or not. The records of the positive cases were  
107 analyzed. We undertook a survey of the demographic and epidemiologic data, as well as the  
108 symptoms. The diagnosis of giardiasis was the only criterion for being included in the study.  
109 It were not necessary exclusion criteria.

110           The following information were processed: the incidence and prevalence of giardiasis  
111 according to age and to the month of the year, as well as prevalence on the age category,  
112 gender and background. All the patients diagnosed with *Giardia* spp. were included in the  
113 study. History was collected by direct interview or, for children, from their relatives. The  
114 patients included in the study were separated according to gender and age groups: 0-1 years  
115 old, 1-4, 5-9, 10-15, 15-25, 25-35, 35-45, 45-55, 55-65, 65-74, 75-84, and >85 years old. Full  
116 diagnosis at discharge was also analysed and correlated with *Giardia* infection.

### 117 **2.2. Ethical statement**

118           The study was approved by the Ethics Commission of the Council of the Medicine and  
119 Pharmacy Faculty, University of Oradea, and conducted in accordance with the World  
120 Medical Association Code of Ethics (Declaration of Helsinki, 1967). Each patient included in  
121 the study signed an informed-consent form.

### 122 **2.3. Setting**

123 The current study was carried out in a single tertiary center, namely the Department of  
124 Infectious Disease, Municipal Hospital Oradea “Gavril Curteanu”, Bihor County, Romania.  
125 The Bihor County is located in the north-west of Romania. It has a population of 566,435  
126 (2017), 51% of the population living in urban areas. The Municipal Hospital from Oradea  
127 serves the entire county, both the rural and the urban areas. This hospital admits both children  
128 and adults.

#### 129 **2.4. Diagnosis of giardiasis**

130 The copro-parasitological technique used to diagnose giardiasis was the Willis  
131 method. The Willis technique is considered to be a floatation method, which is based on the  
132 ability of helminth eggs to float on / to the surface of a saturated NaCl solution ( $\rho=1.20$  g /  
133 mL) and to adhere to the glass. By this technique, a saturated solution with emulsified faeces  
134 is introduced into a round bottom flask; a meniscus forms on its surface. The flask is then  
135 covered with a glass blade/slide. After a few minutes, the glass blade/slide is removed and  
136 examined under a microscope (Carvalho et al., 2012). Faecal samples (n=3) from each subject  
137 were collected on alternate days (first, third and fifth day), in a sterile universal container,  
138 with no preservative solution. The samples were kept at a temperature of 4 °C and were  
139 processed within 24 hours of collection.

#### 140 **2.5. Statistical analysis**

141 The statistical analysis and graphical representation were performed using the  
142 software package SPSS (Statistical Package for the Social Sciences), version 26 for Windows.  
143 Today, the program is used in different area like marketing, experimental research, education,  
144 health. SPSS use different procedures like descriptive, comparative, reductive, modeling  
145 which some we use in our article: descriptive, frequency and comparative analysis. Also, Chi  
146 square test was used to calculate p-value and determine the confidence interval. Descriptive  
147 statistics (95% Confidence Interval) were calculated for each variable as appropriate. Mann-

148 Whitney U statistics for two independent variables. A p-value of less than 0.05 was regarded  
149 as statistically significant and a strong relation between independent variables which we  
150 consider in this article.

151

### 152 **3. Results**

#### 153 **3.1. Incidence**

154 During the 14 year interval investigated, 54,623 patients, children and adults were  
155 admitted to the tertiary center included in the study and tested for *Giardia* spp. infection. In  
156 this group, 2,441 patients were diagnosed with giardiasis (**Figure 1**), representing a  
157 prevalence of 4.47%. In the observed period of time, the annual prevalence of the infection  
158 with *Giardia* spp. showed variations (16.36%-0.60%). As it can be observed in Figure 1, the  
159 evolution of the infection prevalence during this interval of 14 years showed a decreasing  
160 trend. A frequent association of giardiasis with acute enterocolitis, gallstone diseases, acute  
161 gastritis and acute hepatitis was observed in patients infected with *Giardia* spp. (**Figure 2**).

162 The incidence of giardiasis in each calendar month can be seen in **Figure 3**. During  
163 the 14 years, over 10% of the cases were recorded in the months June, July and August  
164 (10.20%, 10.65% and 10.49%). During our study, increased values were also recorded in the  
165 months January and February, namely 8.89% and 8.68%. The lowest values were recorded in  
166 October (6.96%), November (6.31%) and December (6.80%). Regarding gender, 61% of the  
167 patients infected with *Giardia* spp. were female, the female-male ratio being 1.5:1 (61%  
168 female, 39% male) (**Figure 4**).

169 In this study, the incidence of giardiasis according to age categories varied greatly.  
170 The incidence of giardiasis was higher in patients from urban areas, in comparison with those  
171 from rural areas. Thus, 788 of the patients from rural areas were diagnosed with giardiasis  
172 (36.30%) and 1,434 patients from urban areas (64.70%), the ratio being 1.8:1 (**Figure 5**).



173 Our research revealed that the incidence of giardiasis according to age categories  
174 varied greatly and there was a direct relation to the age of the patients. Over 60% of the  
175 patients were aged 15-54 years (66.86%). A high incidence was observed in patients aged 15-  
176 24 years: 16.14%; in patients aged 25-34 years: 17.94%; in patients aged 35-44 years:  
177 16.80%; and in patients aged 45-54 years: 15.98%. In the other age categories, the incidence  
178 below 15%, the lowest incidence being 0.66%, in patients under one year of age and 0.16% in  
179 patients with ages over 85 (**Figure 6**).

### 180 **3.2. Clinical presentation**

181 As expected, the gastrointestinal and hepatobiliary symptoms were overwhelming. A  
182 small number of patients presented allergic symptoms. The most frequent complaints were  
183 abdominal pain (69.07%), especially diffuse pain, loss of appetite (71.24%) and sensitivity at  
184 palpation (54.69%), especially in the right hypochondrium, hepatomegaly (46.17%). Other  
185 symptoms were nausea (42.52%), vomiting (25.60%), diarrhea (18.48%), constipation  
186 (8.89%), intestinal distension (7.50%) and erythematous skin rash (11.35%) (**Figure 7**). The  
187 abdominal pain was recorded in 10.67% of the infants, 16.62% of the children were 1-4 years  
188 old, in 19.94% of the teenagers and in 13.23% of the adults aged 55-64 years. The lowest  
189 prevalence of abdominal pain was in patients with ages between 45 and 54 (4.31%) and the  
190 highest was observed in teenagers (19.94%) ( $p < 0.001$ ).

191 The loss of appetite was more frequently observed in 18.14% of the patients aged 15-  
192 24 years, the lowest prevalence being recorded in children aged 5-14 years (5.04%)  
193 ( $p < 0.001$ ). Nausea was reported by nearly 8.04% of the patients. Vomiting was reported by  
194 4.84% of the patients, especially in children aged 1-4 years. Hepatomegaly was observed in  
195 nearly 9% of the patients (8.73%). We mention that hepatomegaly was more frequent in  
196 adults. Concerning the intestinal transit disorders, diarrhea was more frequent than  
197 constipation in the patients that participated in the study. Diarrhea was observed in all age

198 groups, the highest percent being represented by infants (50%) ( $p < 0.001$ ). Constipation was  
199 recorded in 4% of the infants, in 5.90% of the children aged 1-4 years and 6.06% in the  
200 elderly ( $p < 0.001$ ). Allergic skin rash appeared in only 5% of the cases, the highest percentage  
201 being observed in children aged 1-4 years (12.06%) ( $p < 0.001$ ). In the other age groups, the  
202 percent varied between 0% and 3.55% (**Figure 8**).

203 All patients with positive tests for *Giardia* were treated according to guidelines with  
204 albendazole; 1,147 (47%) of them returned for follow-up and showed negative tests.

205

#### 206 **4. Discussion**

207 Human giardiasis with universal spread is characterized by epidemics in some  
208 countries, and the spread affects between 1% and 27% of the population (Fletcher et al.,  
209 2014). The incidence of the infection with *G. duodenalis* is lower in developed countries, and  
210 recent studies show values of 1% in Germany, 3.7% in Portugal, 1.30% in England, 1.40 and  
211 5.8% in the United States, 2.50% in North Korea, 6.75% in China (Sagebiel et al., 2009;  
212 Almeida et al., 2006; Davies et al., 2009; Church et al., 2010; Painter et al., 2015; Huh et al.,  
213 2009; Liu et al., 2014). In developing countries, including Romania, the disease has an  
214 extremely high prevalence in children enrolled in communities (pre-schools, kindergartens,  
215 schools), easily transmitting the infection to the other members of the family. The prevalence  
216 of giardiasis in the world, estimated at 7.2% in 1965, fluctuates greatly and depends on  
217 various factors such as age (the highest incidence between the ages 1-3, as high as 85%),  
218 environmental background (more frequent in communities, 5.4%-78%, as opposed to the  
219 family environment 1.5%-21%), the social economical and geographic area (Gillon and  
220 Ferguson, 1984).

221 In Romania, the incidence of infection in humans varies from 15% to 44%, in  
222 communities rarely reaching 37-77%. According to other authors, *Giardia* spp. can affect all

223 age groups, the prevalence of the disease in the world varying between 2% and 25%, being  
224 higher in urban communities, especially in very young children (Steriu, 2003).

225 A correct diagnosis of giardiasis is relevant for the prevention and / or treatment of diseases  
226 that are caused by this helminth. The diagnosis resulting from the laboratory analyses, in the  
227 case of *Giardia* spp., is based mainly on finding and identifying microscopic cysts in faecal  
228 samples. In addition, immunological-based tests along with molecular methods are used for  
229 diagnosis and / or for advanced research. These diagnostic methods exhibit different levels of  
230 sensitivity and specificity. The determination of the pathology itself depends on several  
231 factors, of which can be mentioned: the chosen test method, the ability to carry out the stages  
232 of the identification / detection operations, the skill of operations, as well as the stage in  
233 which the test was performed (Elmi et al., 2017). There is no known traditional or ultra-  
234 modern method able detect absolutely all cases of *Giardia* infection. In the last three decades,  
235 several immunodiagnostic tests (for the rapid diagnosis of giardiasis) based in particular on  
236 the detection of *Giardia* antigens in faecal samples have been developed. At the global level,  
237 due to the high sensitivity of these methods, examination of stool samples by microscopy  
238 (after using the concentration method) is the most frequent procedure performed in laboratory  
239 analysis dedicated to giardiasis. The results obtained are relevant due to their good  
240 performance in the diagnostic strategy. These methods should thus be viewed as the gold  
241 standard (Hooshyar et al., 2019; Soares and Tasca, 2016). In the present study we used the  
242 Willis technique since it is commonly used for detecting light structures (protozoan cysts,  
243 hookworm eggs) (Cerqueira et al., 2007). To increase clinic sensitivity test, 3 samples were  
244 collected on alternative days and then analysed (Elmi et al., 2017).

245 Our study showed a prevalence of 4.47% in a community admitted in a hospital for  
246 infectious diseases. The data are similar to those of Steriu (Steriu, 2003), reported from other  
247 Romanian counties, who state that the infection with *Giardia* spp. has an incidence of 2-25%,

248 respectively 4-7%, but also similar to the data collected by Basualto (Basualto et al., 2007)  
249 and Horteş and Gurvith (2011) who reported an incidence of giardiasis of 6.9%, respectively  
250 1-11%. In a study conducted on hospitalized patients in the south-west of Romania (Neghina  
251 et al., 2011) it was showed an incidence of *Giardia* spp. of 7.80%. The studies conducted by  
252 Akdemir and Helvacı (2007) on a group of 675 people over 15 years of age from Kutahya,  
253 Turkey, the study conducted by Degirmenci et al. (2007), on a group of 3,925 patients from  
254 the University Hospital from Ege, Turkey and the studies conducted by Yaman et al. (2008)  
255 on a group of 28,911 patients that appeared in the Parasitology Laboratory of the University  
256 of Medicine from Erciyes, Turkey, reported much lower incidence of *Giardia* spp., namely  
257 1.48%, 1.78% and 1.96%. Our study found a substantial decline of the infection during the  
258 period in which the patients with *Giardia* spp. were studied. The highest value was reached in  
259 the year 2004 (16.36%) and the lowest in the years 2016 (0.65%) and 2017 (0.69%). In the  
260 last two years we observed much lower values of the prevalence of giardiasis. A decline trend  
261 of the infection with *Giardia* spp. was also observed in the studies conducted by Painter  
262 (Painter et al., 2015), in a retrospective study in the United States and in the studies conducted  
263 by Jejaw (Jejaw et al., 2014), in a retrospective study in Jumma Town, Ethiopia, with an  
264 incidence of the *Giardia* spp. infection lower than 5%.

265 In our study, the monthly prevalence of giardiasis varies between 10.65% and 6.31%,  
266 the highest percent being recorded in the months June, July and August (10.20%, 10.65% and  
267 respectively 10.49%), and the lowest percent in the months October, November and  
268 December (6.96%, 6.31% and respectively 6.80%).

269 Painter et al. (2015), in a study conducted in the United States, observed higher  
270 incidences of the infection during summer. A study conducted by Terletskaia-Ladwig  
271 Terletskaia-Ladwig et al. (2011), in Germany, reported an incidence of 15% of infection with  
272 *Giardia* spp. from June until August, but disproves the incidence on the infection in October

273 and November. Mbae et al. (2013), in a two years study, conducted on children with diarrhea  
274 also observed a high incidence of the infection in the months of June and August, but also  
275 from September until November, a period in which our study showed lower incidences. The  
276 high incidence of infection with *Giardia* spp. in humans during the summer months could be  
277 explained by the connection between the consumption of contaminated water, the frequency  
278 of travelling and time spent outside, which is a factor for transmitting the cysts of *Giardia*  
279 spp. in humans.

280         Based on the gender, the data presented in our study revealed a higher incidence in  
281 females, namely 61% in females and 39% in males. The higher prevalence of the infection  
282 with *Giardia* spp. in females as opposed to that in males, as showed in our study, is in key  
283 with some previous studies (Wansaas et al., 2009; Persson et al., 2015). Other studies  
284 conducted on both adults and children show different data from ours. According to the studies  
285 conducted by Ostan (Ostan et al., 2007) on a group of 294 children, by Kassem et al. (2007)  
286 on a group of 350 children and newborn babies and by Warrunee et al. (2007) on 1920  
287 children show no significant differences regarding the gender of the people infected with  
288 *Giardia* spp. Also, a series of previous studies show a higher incidence in males than in  
289 females (Wansaas et al., 2009; Mellingen et al., 2010; Escobedo et al., 2011; Asher et al.,  
290 2014; Ramos et al., 2014; Tamer et al., 2008; Tamer et al., 2015).

291         The data we collected in our study show a higher incidence of infection with *Giardia*  
292 spp. in people who come from urban areas, than in people from rural areas (63.70%,  
293 compared to 36.30%). Our data are similar to those presented by Neghina et al. (2013), in a  
294 study conducted in Timis County, Romania, on a 14 years period of time. A study conducted  
295 by Korzeniewski et al. (2014), on children and adults in a provincial hospital in Afghanistan  
296 showed an incidence of infection with *Giardia* spp. of 38.3%, these data being similar to the  
297 ones we have found. According to Al-Aghra and Teodorescu, in a study conducted in

298 Palestine, the incidence of the infection with the *G. intestinalis* in people from urban areas is  
299 33%, and 53% in people from urban areas (Al-Aghra and Teodorescu, 2000). Studies  
300 conducted by Basualdo et al. (2007) in Argentina and by Quattara et al. (2008) in France on  
301 individuals from rural areas, have showed much lower incidences of infection with *Giardia*  
302 spp. (18.1% and 17.5%). The high prevalence of the infection in urban areas can be due to the  
303 poor sanitation in some of the poor areas of the city, the access to drinking water and the poor  
304 hygiene.

305 In our study, the intestinal infection with *Giardia* spp. can be associated with age, the  
306 highest incidence of the infection being in the age groups 5-14 years old (13.40%), 15-24  
307 years old (16.14%), 25-34 years old (17.94%), 35-44 years old (16.80%) and 45-54 years old  
308 (15.98%). They are significantly higher than in other age groups (there is an incidence of  
309 0.66% in infants and an incidence of 3.66% in children aged 1 to 4 years old). The number of  
310 cases of infection with *Giardia* spp., higher in adults and teenagers, could be explained by the  
311 fact that more adults than children came to the hospital. The data from our study regarding the  
312 incidence of *Giardia* spp. infection in teenagers and adults is disproved by Degirmenci et al.  
313 (2007) and Yamon et al. (2008) in studies conducted in Turkey, which reported a much lower  
314 percent (namely 1.78% and 1.96%). In the United States, Painter et al. (2015) observed the  
315 highest incidence in children aged 1 to 4 years old (16.4%), followed by children aged 5 to 9  
316 years old (8.4%) and a much lower percent in adults. Yoder et al. (2010), in a study conducted  
317 over a period of 3 years, observed the highest number of cases of infection with *G. duodenalis*  
318 in children aged 1 to 4 years old. Asher in a study conducted on a native community in  
319 Australia, observed a much higher percent of the infection than the ones reported in our study  
320 (26% in children aged 0 to 5 years old and 30% in children aged 5 to 10 years old), (Asher et  
321 al., 2014). A study conducted by Mbae et al., in Kenya on children with diarrhea, showed an

322 incidence of 1.30% in infants under 1 year of age and a high incidence of infection in children  
323 aged 1 to 5 years old, namely 4.00%, (Mbae et al., 2013).

324 Various studies conducted on children recorded a number of more than 10% of  
325 infection with *G. duodenalis*. Giraldo-Gomez et al. in a study conducted on preschool  
326 children from Armenia, Columbia, observed a prevalence of infection with *G. duodenalis* of  
327 13%. *G. duodenalis* is the most common parasite, (Giraldo-Gomez et al., 2005). Lindo et al.  
328 in a study conducted on children under the age of 12 in a town from Guyana observed an  
329 incidence of infection with *G. duodenalis* of 10.5%, (Lindo et al., 2002). Atas et al. (2008)  
330 observed a prevalence of 15.5% of infection with *G. intestinalis* in children from two schools  
331 in Turkey. Ostan et al. (2007) in a study conducted in Turkey, observed an incidence of  
332 infection of 13.7%, and Quadros et al. (2004) in a study conducted in Brazil, observed an  
333 incidence of infection with *G. duodenalis* of 14%. Other studies have showed an incidence of  
334 infection with *G. duodenalis* of less than 10% (Astal, 2004; Celik et al., 2006; Aksoy et al.,  
335 2007; Worku et al, 2009; Tamer et al., 2008; Hamamci et al., 2011).

336 Regarding the clinical significance of the infection with *Giardia* spp. in the subjects  
337 that have participated in the study, we have tried to describe the association of the clinical  
338 signs with the presence of the parasitic infection, with the mention that some of the subjects,  
339 beside the parasitic infection, also suffered from other diseases that could interfere with the  
340 clinical manifestations of the *Giardia* infection. It is well known that the infection with *G.*  
341 *duodenalis* can progress with many acute or chronic clinical manifestations, or it can be  
342 asymptomatic (Pierce and Kirkpatric, 2009). Our priority was not to establish the exact  
343 clinical symptoms exhibited by the patients who have an infection with *Giardia* spp.  
344 Regardless of the presence of another disease associated with the *Giardia* spp. infection, we  
345 observed a high incidence of abdominal pain (69.07%), lack of appetite (71.24%), sensitivity  
346 of the abdomen (54.69%), especially in the right hypochondrium, and hepatomegaly

347 (47.17%), and a lower incidence of clinical symptoms such as diarrhea (18.48%), nausea  
348 (42.52%), vomiting (25.60%) and erythematous skin rash (11.35%). It has also been observed  
349 that the clinical manifestations associated with the parasitic infection have a higher frequency  
350 in adults and teenagers than in infants and small children, as Mellingen et al. (2010) also  
351 observed in a study conducted in Norway on preschool children, in which he states that the  
352 clinical manifestations of the children infected with *G. duodenalis* are limited. The lack of  
353 appetite has been observed in all the studied age groups, but more frequently found in adults  
354 and having a lower incidence in small children, where the basic manifestation was diarrhea.  
355 This disproves the studies conducted by Ignatius on 583 children under the age of five in  
356 Rwanda (Ignatius et al., 2012). In our study, the abdominal pain was frequently reported in  
357 both children and adults infected with *Giardia* spp., a fact observed also in the studies  
358 conducted by Younas in Pakistan, on 239 children with recurrent abdominal pain (Younas et  
359 al., 2008). The same thing has been noticed in Escobedo's retrospective study on hospitalized  
360 children in a paediatric hospital in Havana (Escobedo et al., 2011). Diarrhea or constipation  
361 have been observed in a small percentage of the subjects that have participated in the study  
362 (18.48% and 8.89%), which suggests that giardiasis can evolve, in certain cases, without  
363 changes in the intestinal transit. In the previous studies, diarrhea is the most common clinical  
364 manifestation that was observed in people infected with *G. duodenalis* (El Guamri et al.,  
365 2009; Escobedo et al., 2011) data consistent with our data. Among the rarest clinical  
366 manifestations of the infection with *G. duodenales*, there are also the allergic manifestations,  
367 previous studies showing the possibility of associating the parasitic infection with the  
368 occurrence of allergic manifestations (Escobedo et al., 2011; Nenoff et al., 2006). A possible  
369 association has also been observed in our study, as 11.35% of the infected subjects presented  
370 erythematous skin rash, with the mention that we established no other cause for the skin rash.



371           The decrease in the prevalence of the infection with *Giardia* spp. observed in our  
372 study can be explained by improved hygienic dwelling conditions, both in urban and rural  
373 areas, associated with better personal hygiene. The persistence of the parasite is explained by  
374 its natural resistance to the processing of drinking water, precarious hygienic conditions in  
375 some dwellings, cohabitation with farm animals, contact with pets or street dogs.

376           The advanced technology and modern methods used for detecting and monitoring  
377 pathogens from different waters, obviously, ensure a better monitoring of them. The cysts  
378 from *Giardia* were detected from numerous environmental matrices (hydrographic basins that  
379 provide source of drinking water, the waters used for recreational activities, soil, sewage,  
380 sludge, etc.), independent of the economic income of the area in which these types of water  
381 are located. Water from the environment, regardless of its origin and use, is extremely  
382 vulnerable to contamination with *Giardia*, due to leakage to the ground and the uncertain  
383 wastewater disposal module, as varied as the source (industrial, agricultural, zootechnical,  
384 household, etc.). At this dispersion, major and frequent infestation, and transmission, a major  
385 role is played by faeces excreted by a large number of hosts (humans, animals), but also by  
386 the increased ability of cysts to survive in the environment, under extremely precarious  
387 conditions. The legislation and policies of many countries ensure compliance with the  
388 parameters for the quality of the source water and the protection of the environment; however,  
389 *Giardia* persists in being a major cause of the formation of outbreaks of pathologies  
390 transmitted through water. As an real evidence, correct knowledge and according to the  
391 reality of the way of contamination of the environment by cysts, the data on the occurrence,  
392 persistence, their survival, can optimize the understanding of the epidemiology and contribute  
393 major to the establishment of priorities, respectively of preventive measures to ensure good  
394 public health.

395

396 **5. Conclusion**

397 *Giardia* spp. is one of the most common aquatic parasites that infect humans and  
398 animals alike. Data collected between 2004 and 2017 show an increased incidence of  
399 infection with *Giardia* spp. in both children and adults hospitalized in the Department of  
400 Infectious Disease, in the West of Romania. Diarrhea, epigastric cramps, flatulence, nausea,  
401 vomiting and weight loss are the main symptoms of human acute giardiasis. To take action in  
402 reducing the cases of giardiasis, it is necessary to evaluate the exact factors that influence or  
403 cause the disease, followed by the identification or implementation of methods to reduce the  
404 effects of the identified factors. In this regard, intervention programs need to be developed  
405 based on systematic studies, programs and politics which must be implemented through an  
406 interdepartmental and interinstitutional synergic cooperation.

407

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414

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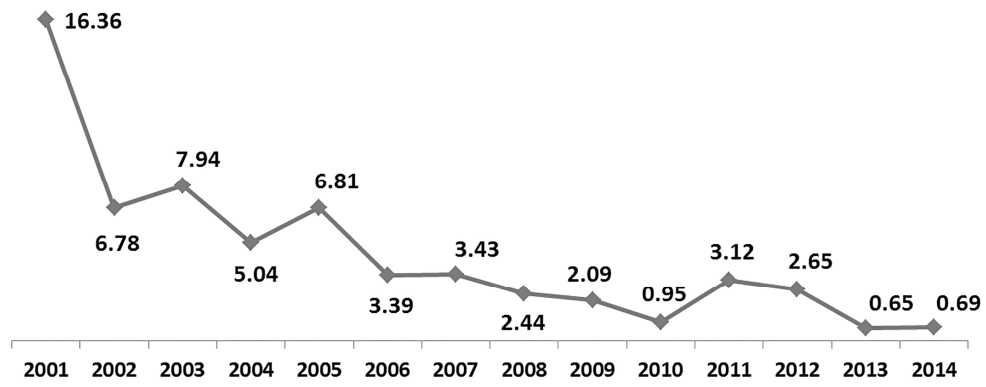


Figure 1

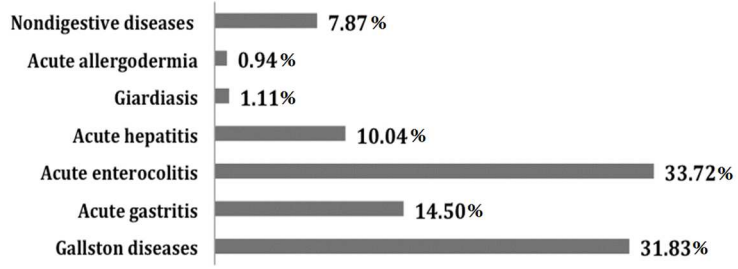


Figure 2

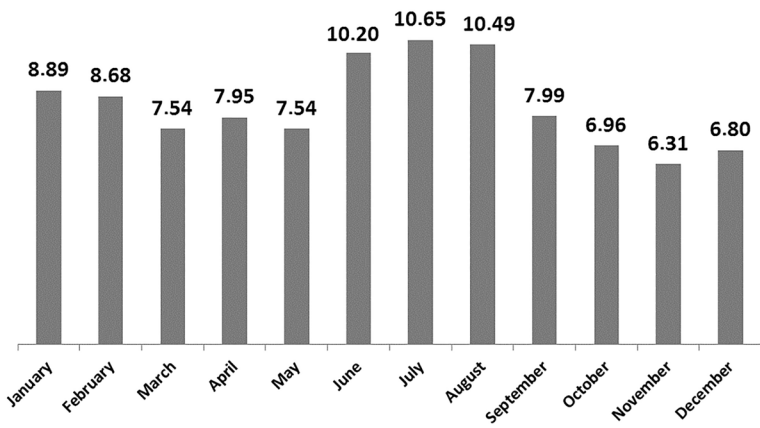


Figure 3

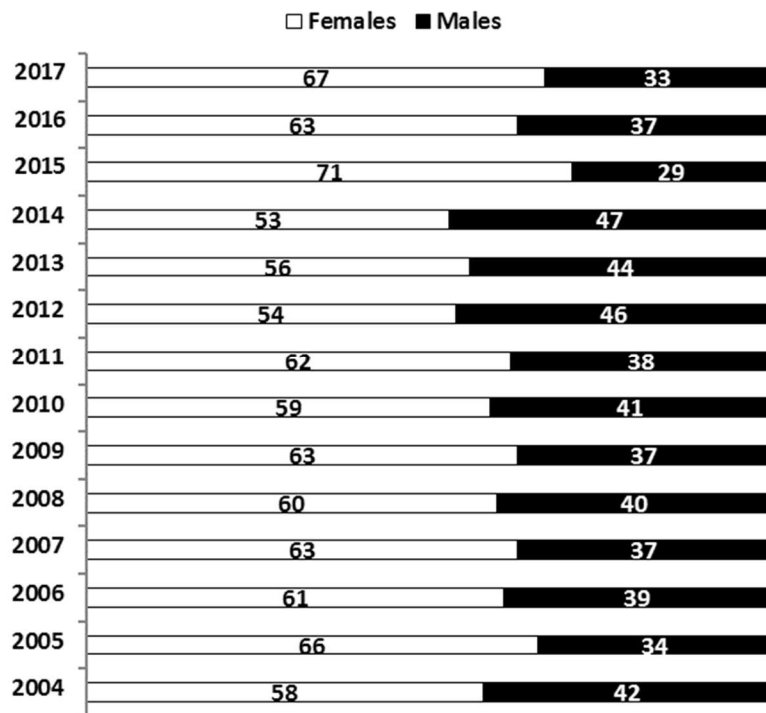


Figure 4

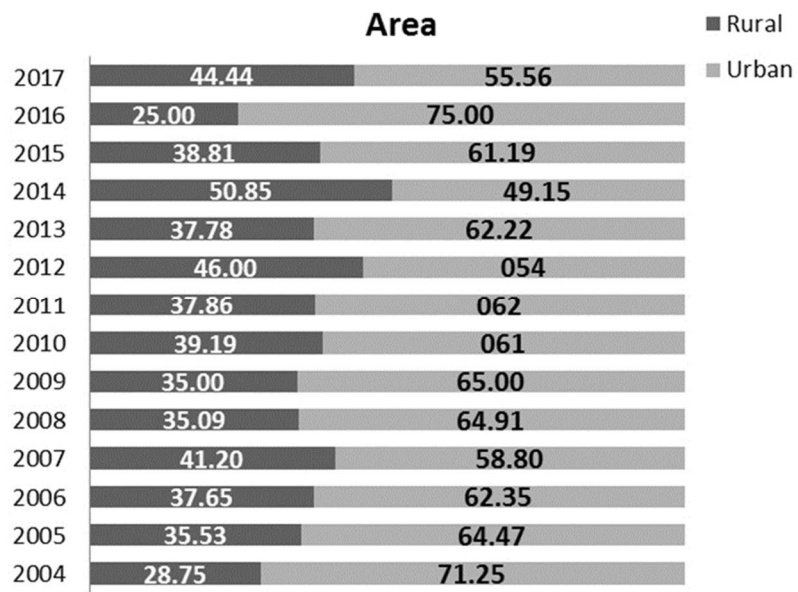


Figure 5



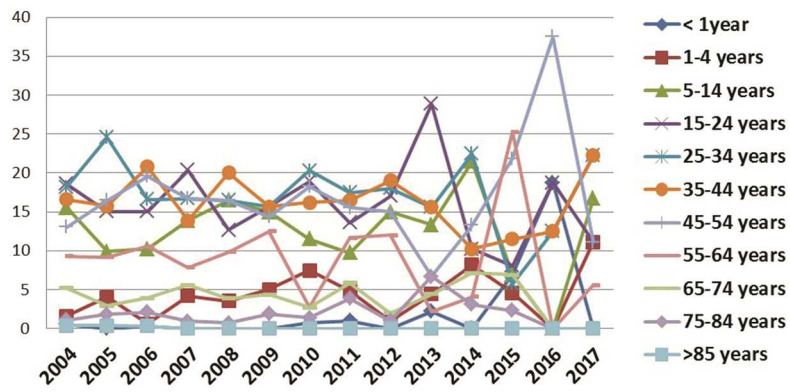


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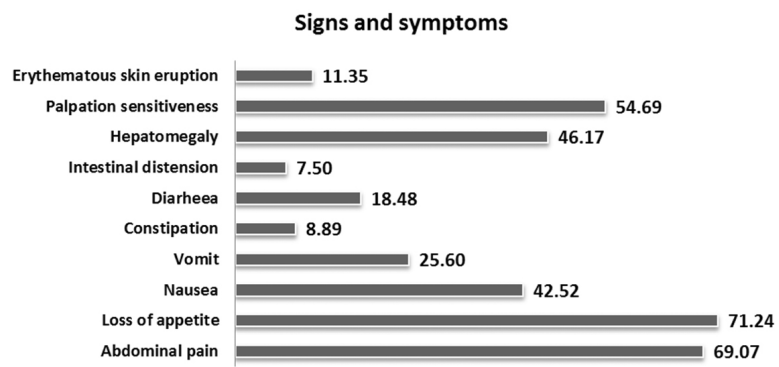


Figure 7

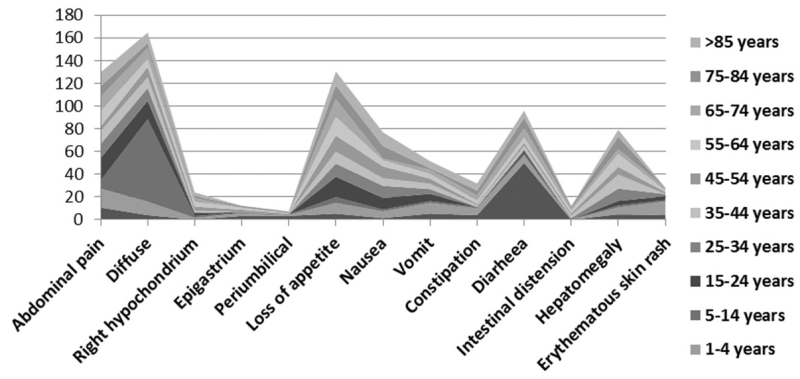


Figure 8