

Epidemiology of human giardiasis in Romania: A 14 years survey

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26

27 Abstract

28 *Giardia* spp. is the most common intestinal protozoan (causing gastrointestinal illness) 29 and the most frequent cause of parasitic gastroenteritis in humans and animals worldwide. 30 The aim of this study was to highlight new data in a specific area regarding clinical 31 presentation and epidemiological prevalence over a long period of time. Patients (a total 32 number of 54,623 patients) admitted in a tertiary center for infectious diseases serving a 33 county for a period of 14 years were tested for infection with Giardia. Positive cases were 34 recorded through analyzing the clinical complaints, the month of incidence and the 35 demographic area from which the patients came from. Longitudinal trends have been 36 evaluated. The incidence of giardiasis among the tested patients was 4.47%. A decreasing 37 trend was observed regarding the annual incidence. Patients between the ages of 15 and 44 38 presented most commonly giardiasis, especially those from urban areas and women. The most 39 common symptoms are loss of appetite (71.24%) and abdominal pain (69.07%). The highest 40 monthly incidence was quoted in July (10.65%), August (10.49%) and June (10.20%). This 41 epidemiological study allows a better knowledge of the infection with Giardia spp. It gives 42 the long-term changes in demographic characteristics of the infected patients in a specific area 43 and the monthly incidence.

44

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

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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 approximately 2.8×10^8 new cases emerging every year (Lanes and Lloyd, 2002). G. lamblia is 54 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 (Sărmăşan 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).

It can manifest in endemic form, especially in tropical and subtropical areas, or in an 74 epidemic form (that affects the travelers in the endemic areas, as it is believed that 2-3% of 75 the travelers' diarrheas are caused by Giardia spp.). It can also occur in restricted 76 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, 2014). The extra-digestive symptoms are also an important part of the clinical panel of 95 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.

The following information were processed: the incidence and prevalence of giardiasis according to age and to the month of the year, as well as prevalence on the age category, gender and background. All the patients diagnosed with *Giardia* spp. were included in the study. History was collected by direct interview or, for children, from their relatives. The patients included in the study were separated according to gender and age groups: 0-1 years 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 diagnosis at discharge was also analysed and correlated with *Giardia* infection.

117 **2.2. Ethical statement**

The study was approved by the Ethics Commission of the Council of the Medicine and Pharmacy Faculty, University of Oradea, and conducted in accordance with the World Medical Association Code of Ethics (Declaration of Helsinki, 1967). Each patient included in 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 (ρ =1.20 g / 133 mL) and to adhere to the glass. By this technique, a saturated solution with emulsified faeces is introduced into a round bottom flask; a meniscus forms on its surface. The flask is then 134 135 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**

The statistical analysis and graphical representation were performed using the software package SPSS (Statistical Package for the Social Sciences), version 26 for Windows. Today, the program is used in different area like marketing, experimental research, education, health. SPSS use different procedures like descriptive, comparative, reductive, modeling which some we use in our article: descriptive, frequency and comparative analysis. Also, Chi square test was used to calculate p-value and determine the confidence interval. Descriptive statistics (95% Confidence Interval) were calculated for each variable as appropriate. MannWhitney U statistics for two independent variables. A p-value of less than 0.05 was regarded as statistically significant and a strong relation between independent variables which we 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).

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

In this study, the incidence of giardiasis according to age categories varied greatly. The incidence of giardiasis was higher in patients from urban areas, in comparison with those from rural areas. Thus, 788 of the patients from rural areas were diagnosed with giardiasis (36.30%) and 1,434 patients from urban areas (64.70%), the ratio being 1.8:1 (**Figure 5**). Our research revealed that the incidence of giardiasis according to age categories varied greatly and there was a direct relation to the age of the patients. Over 60% of the patients were aged 15-54 years (66.86%). A high incidence was observed in patients aged 15-24 years: 16.14%; in patients aged 25-34 years: 17.94%; in patients aged 35-44 years: 16.80%; and in patients aged 45-54 years: 15.98%. In the other age categories, the incidence below 15%, the lowest incidence being 0.66%, in patients under one year of age and 0.16% in 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).

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**).

All patients with positive tests for *Giardia* were treated according to guidelines with 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).

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

age groups, the prevalence of the disease in the world varying between 2% and 25%, being
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 of the identification / detection operations, the skill of operations, as well as the stage in 232 233 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).

Our study showed a prevalence of 4.47% in a community admitted in a hospital for infectious diseases. The data are similar to those of Steriu (Steriu, 2003), reported from other 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 Hortez 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 Helvaci (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%.

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

Painter et al. (2015), in a study conducted in the United States, observed higher incidences of the infection during summer. A study conducted by Terletskaia-Ladwig Terletskaia-Ladwig et al. (2011), in Germany, reported an incidence of 15% of infection with *Giardia* spp. from June until August, but disproves the incidence on the infection in October and November. Mbae et al. (2013), in a two years study, conducted on children with diarrhea also observed a high incidence of the infection in the months of June and August, but also from September until November, a period in which our study showed lower incidences. The high incidence of infection with *Giardia* spp. in humans during the summer months could be explained by the connection between the consumption of contaminated water, the frequency of travelling and time spent outside, which is a factor for transmitting the cysts of *Giardia* 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 with some previous studies (Wansaas et al., 2009; Persson et al., 2015). Other studies 283 284 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 Kasssem 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).

The data we collected in our study show a higher incidence of infection with *Giardia* spp. in people who come from urban areas, than in people from rural areas (63.70%, compared to 36.30%). Our data are similar to those presented by Neghina et al. (2013), in a study conducted in Timis County, Romania, on a 14 years period of time. A study conducted by Korzeniewski et al. (2014), on children and adults in a provincial hospital in Afghanistan showed an incidence of infection with *Giardia* spp. of 38.3%, these data being similar to the ones we have found. According to Al-Aghra and Teodorescu, in a study conducted in Palestine, the incidence of the infection with the *G. intestinalis* in people from urban areas is 33%, and 53% in people from urban areas (Al-Aghra and Teodorescu, 2000). Studies conducted by Basualdo et al. (2007) in Argentina and by Quattara et al. (2008) in France on individuals from rural areas, have showed much lower incidences of infection with *Giardia* spp. (18.1% and 17.5%). The high prevalence of the infection in urban areas can be due to the poor sanitation in some of the poor areas of the city, the access to drinking water and the poor 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 cases of infection with Giardia spp., higher in adults and teenagers, could be explained by the 310 311 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

incidence of 1.30% in infants under 1 year of age and a high incidence of infection in children
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 of the abdomen (54.69%), especially in the right hypochondrium, and hepatomegaly 346

(47.17%), and a lower incidence of clinical symptoms such as diarrhea (18.48%), nausea 347 (42.52%), vomiting (25.60%) and erythematous skin rash (11.35%). It has also been observed 348 349 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 association has also been observed in our study, as 11.35% of the infected subjects presented 369 370 erythematous skin rash, with the mention that we established no other cause for the skin rash.

The decrease in the prevalence of the infection with *Giardia* spp. observed in our study can be explained by improved hygienic dwelling conditions, both in urban and rural areas, associated with better personal hygiene. The persistence of the parasite is explained by its natural resistance to the processing of drinking water, precarious hygienic conditions in 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

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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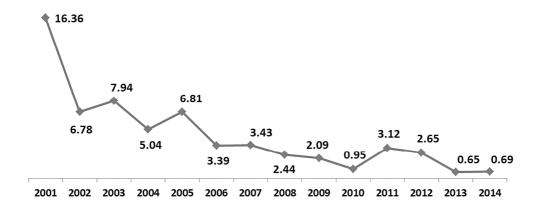
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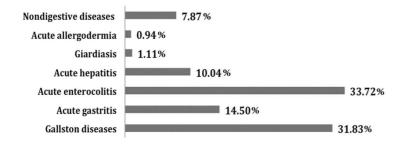
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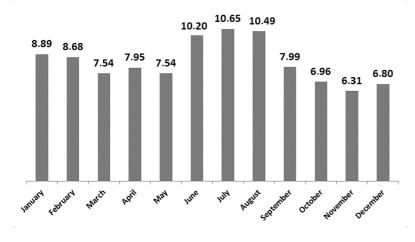
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- 618 **Figure 1.** Prevalence of *Giardia* spp. infection according to year
- 619 Figure 2. Diseases associated with *Giardia* spp. infection
- 620 **Figure 3.** Distribution of cases for *Giardia* spp. infection according to month
- 621 Figure 4. Prevalence of *Giardia* spp. infection according to year and gender
- 622 **Figure 5.** Prevalence of *Giardia* spp. infection according to year and demographic area
- 623 Figure 6. Distribution of cases for *Giardia* spp. infection according to age and year
- 624 **Figure 7.** Symptomatology of *Giardia* spp. infection in patients
- 625 Figure 8. Symptomatology of *Giardia* spp. infection according to age

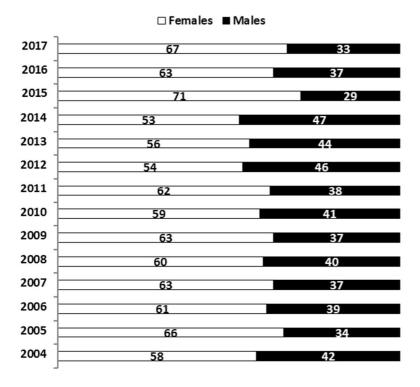












Area			
2017	44.44	55.56	🔳 Urban
2016	25.00	75.00	
2015	38.81	61.19	
2014	50.85	49.15	
2013	37.78	62.22	
2012	46.00	054	
2011	37.86	062	
2010	39.19	061	
2009	35.00	65.00	
2008	35.09	64.91	
2007	41.20	58.80	
2006	37.65	62.35	
2005	35.53	64.47	
2004	28.75	71.25	

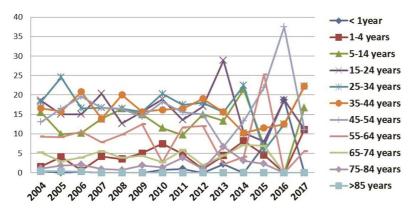
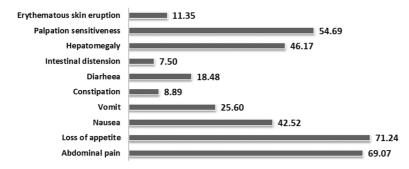


Figure 6

Signs and symptoms



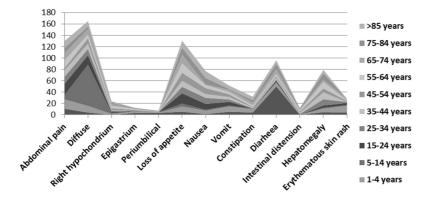


Figure 8