

Assessment of *Giardia lamblia* Infection Prevalence Using Lugol's Iodine Staining Technique

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Parasitic diseases have a worldwide distribution and represent an important public health problem. The aim of the present study was to evaluate the prevalence of intestinal parasitic infections in a Child Day Care Center from Western Romania using direct wet mount and concentration procedures of stool samples. One hundred sixty-four children aged 2-8 years were investigated. Stool examinations were performed using the iodine staining for the identification of protozoan cysts and the Willis-Hung concentration method for the identification of helminth eggs. Routine laboratory investigations were also conducted to evaluate the eosinophil count in the infected children. Intestinal parasitic infections were diagnosed in 47 cases (28.6%). Giardia lamblia (25%), Entamoeba coli (5.5%), Blastocystis hominis (1.2%), Ascaris lumbricoides (0.6%) and Trichuris trichiura (0.6%) were the only parasites identified. Among the children with parasitic infections we have determined association of two parasites in 7 (14.9%). We have found that in children with giardiasis the eosinophil values were increased (5.54±4.21%) compared to controls (3.69±1.48%) (p<0.01). Our findings suggest that giardiasis may be significantly prevalent in children attending day care centers in Western Romania. Efficient educational programs should be timely implemented in child care centers with the aim of providing appropriate infection control measures.

Keywords: iodine staining, concentration methods, giardiasis, protozoan, parasite

Giardia lamblia (synonymous with *Giardia duodenalis* and *Giardia intestinalis*) is an enteric flagellated protozoan that causes giardiasis, one of the most commonly reported intestinal parasitoses in humans, with an estimated 2.8 million new cases per year [1]. This parasite is endemic throughout the world and is one of the most frequently found intestinal parasites in children living in developing countries [2]. Transmission of *Giardia* is via the faecal-oral route, either through contaminated water or food, or from person to person [3]. These routes of transmission occur more frequently in day-care centers, where children are in close contact and poor hygiene may be practiced [1].

In Romania, giardiasis is the most commonly reported intestinal parasitic infection with the highest incidence in patients aged 0-14 years [2]. Protozoal colonization of the intestinal tract is common in institutionalized Romanian children and may play a role in causing morbidity and mortality in this high-risk group of children [4].

The aim of the present study was to determine the prevalence of intestinal parasitic infections in a Child Care Center from Western Romania. Laboratory investigations were also conducted to evaluate symptoms and eosinophil count in the infected children.

Experimental part

This study was conducted in a Child Care Center from Arad, Romania, with a population of 159704. Members of the personnel collected stool samples, for parasitological examination, from all 164 children residing in the child care center. Stools were obtained from each child on three non-consecutive days within two weeks. The stool specimens were collected in labeled sterile plastic vials without preservatives and transported to the laboratory. They were examined for the presence of parasites by direct wet mount and concentration techniques at the Discipline of

Parasitology of Victor Babes University of Medicine and Pharmacy, in Timisoara, Romania. Stool examinations were performed using the iodine staining for the identification of protozoan cysts and the Willis-Hung concentration method for the identification of helminth eggs. All preparations were examined microscopically at 400x for 5 to 10 min, respectively.

Laboratory investigations were also conducted to evaluate the eosinophil count in the infected children. Eosinophil values were determined in the peripheral blood by differential white blood cell count with May-Grunwald-Giemsa staining. An eosinophil count of >4% was considered in normal range. The control group consisted of 58 healthy children age matched.

Statistical Methods

Data was entered in a Microsoft Excel database, version 2011 (Microsoft Corp, Redmond, WA, USA), and univariate analyses performed with Epi Info statistical package 3.3.2 (Centers for Disease Control and Prevention, Atlanta, GA). Mantel-Haenszel chi-square and Fisher's exact test 2-tailed were used for comparison between groups. A p-value of < 0.05 was considered to indicate significance.

Results and discussions

The 164 institutionalized children ranged in age from 2 to 8 years (mean = 3.8 years), 93 (56.7%) were females. Intestinal parasitic infections were diagnosed in 47 (28.6%) of the 164 investigated children. *Giardia lamblia* 41 (25%), *Entamoeba coli* 9 (5.5%), *Blastocystis hominis* in 2 (1.2%), *Ascaris lumbricoides* in 1 (0.6%) and *Trichuris trichiura* in 1 (0.6%) were the only parasites identified (table 1). *Giardia lamblia* was diagnosed in 41 (87.2%) of 47 infected children. Among the children with parasitic infections we have determined association of two parasites in 7 (14.9%) (table 1). Twenty-three (32.4%) of 93 girls and 18 (25.3%)

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Parasites	N	%	Infection category	N/ %
<i>Giardia lamblia</i>	36	21.9	Monoinfection	40/ 85.1
<i>Entamoeba coli</i>	4	2.4		
<i>Blastocystis hominis</i> + <i>Entamoeba coli</i>	2	1.2	Coinfection	7/ 14.9
<i>Giardia lamblia</i> + <i>Entamoeba coli</i>	3	1.8		
<i>Giardia lamblia</i> + <i>Ascaris lumbricoides</i>	1	0.6		
<i>Giardia lamblia</i> + <i>Trichuris trichiura</i>	1	0.6		
Total children diagnosed / investigated	47/ 164	28.6	-	47/ 100

Table 1
PARASITIC INFECTIONS
DIAGNOSED IN 164 CHILDREN

(Legend: N = number of cases)

of 71 boys were infected with *G. lamblia*. However, there was no significant gender difference in the children who excreted *G. lamblia* cysts.

We have found that in children infected with *G. lamblia*, the eosinophil values were increased ($5.54 \pm 4.21\%$) compared to controls ($3.69 \pm 1.48\%$) ($p < 0.01$). Laboratory results revealed that 22 (61%) of the 36 children $\leq 4\%$.

Day care centers are special environments where close clustering of children and staff, promotes the passage of microorganisms between individuals. The microorganisms, once introduced into a day care unit by a source child, are spread to other children and staff members, and are deposited on environmental surfaces which may serve as possible sources for further transmission [5, 6].

In the present study, results revealed a 28.6% prevalence of parasitic infections diagnosed in institutionalized Romanian children. Seven (14.9%) of the 47 infected children had association of two parasites. *G. lamblia* was the most frequently identified parasite (25%). This prevalence is significantly higher than those reported in similar children settings in two recent European studies [7, 8]. In Germany, 5.5% of the children in one kindergarten were found infected with *G. lamblia* [7] and in the United Kingdom, the prevalence of *Giardia* carriage among 230 asymptomatic preschool children attending day-care facilities was 1.3% [8]. However, *G. lamblia* prevalence in this study was slightly lower than the prevalence of 25.3% reported in children attending regularly day care centers in Spain [9].

In Romania, giardiasis was found to be common in institutionalized children [10,11,13]. In a study performed in Bucharest in 1991, *G. lamblia* was identified in 72% of the fecal specimens examined in institutionalized children [4]. High prevalence rates of giardiasis have also been demonstrated among other groups of institutionalized Romanian children [10, 12]. For instance, Panaitescu and colleagues diagnosed *G. lamblia* infections in 18.1% of the children with physical and psychic handicaps [10] and Barabas et al identified *G. lamblia* cysts in 12.6% of the children hospitalized in a tuberculosis facility in Mures County, Romania [12].

Giardiasis was also frequently reported in non institutionalized Romanian children. Rozen and coworkers determined a prevalence of 16.9% for giardiasis in children aged 3-5 years from an urban population [14]. In a 14-year retrospective study, we have recently reported the highest incidence of giardiasis in Romanian patients aged 0-14

years, with a higher mean annual incidence in urban inhabitants [2].

As previously reported by others, the present study indicated that *G. lamblia* prevalence was not significantly different between males and females [7].

Conclusions

Our findings suggest that giardiasis may be significantly prevalent in Romanian Child Care Centers. Crowded living conditions, children's poor hygiene and their immunologic susceptibility to infectious processes may facilitate transmission [4, 15]. The contamination of hands and common toys as well as a lack of infection control measures may play a role in the transmission of this protozoan in day care centers. Exposure to feces through handling diapers and poor hygiene, particularly after toileting, in childcare settings might contribute to increased risk [16, 17].

Additional education and training for children on proper toilet and food hygiene may help to further reduce the possibility of child-to-child transmission [7]. Person-to-person transmission of *G. lamblia* infection is difficult to interrupt, especially in child care settings [16, 18]. Therefore, efficient educational programs should be timely implemented in child care centers with the aim of providing appropriate infection control measures, of which hand washing, diaper changing, separation of ill children and antiparasitic treatment are recommended for controlling giardiasis.

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