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## Prevalence of *Giardia lamblia* in diarrheic children in Almadinah Almunawarh, KSA

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### Abstract

*Giardia lamblia* is one of the most common intestinal parasites of humans. It is a flagellated, teardrop-shaped parasite which has two life forms, the trophozoite and the cyst. This study was carried out in Almadinah Almunawarh in 2011 to determine the prevalence of giardiasis in diarrheic children. Three methods were used in this study: direct microscopic examination of stool samples, formol-ethyl acetate concentration technique and rapid dipstick immunodiagnostic technique (detection of *Giardia* copro-antigen). Out of 889 stool specimens collected from children, giardiasis was detected in 1.9% of them. The age group 6 – 8 years had the highest rate (41.2%) and 4–6 years the lowest (29.4%). Also, males had a higher rate than females (52.9% and 47.1%), respectively. Diagnosis of giardiasis should rely on microscopic examination of stool samples. Concentration techniques are to be done if available, together with sensitive and specific immunochromatographic tests.

**Keywords:** *Giardia lamblia*; giardiasis; children; diarrhea; copro-antigen; Almadinah; K.S.A.

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## Introduction

*Giardia* is an intestinal flagellate protozoan which exists in two stages: an active trophozoite stage and the dormant cyst stage, which is the infective stage [1]. There are three identified species of *Giardia*: *Giardia lamblia*, *Giardia muris*, and *Giardia agilis*. *Giardia lamblia* is the only species of them known to infect humans. Transmission of the cyst, and the disease, occurs mainly by the feco-oral route [1, 2]. It is a cosmopolitan parasite with an overall prevalence rate of 20-30% in developing countries, higher numbers of infections are seen in the late summer months. Travellers to regions of Africa, Asia, and Latin America where clean water supplies are low are at increased risk of contracting the infection [2]. Some healthy people do not get sick from *Giardia lamblia*; however they can still pass the infection on to others. Children, seniors, and people with long-term illnesses may be more prone to contracting the infection as the risk of transmission is higher in day care centres and seniors' residences and in hypogammaglobulinemia patients; which makes it an opportunistic infection [3].

Clinical manifestations are usually diarrheal, abdominal cramps, nausea, bloating and loss of appetite. In chronic and complicated cases, cholecystitis and malabsorption may be observed [4].

The most common way to diagnose giardiasis is by the visualization of the cysts by experienced professionals. The cysts are detected 50-70% of the time in the first stool specimen examined. Over 90% of the time the cysts are detected after 3 stool specimens have been examined. So, more than 1 specimen may be required [4, 5]. Another method of diagnosis that is commonly used as a screening tool is antigen assay of stool samples. This method detects a certain protein found in the wall of *Giardia*. A stool sample is mixed with a solution that detects the cysts in the stool [1, 6]. Molecular techniques may be resorted to for diagnosis in refractory cases [7].

Given the importance of giardiasis as a common cause of diarrhoea, it was decided to investigate its prevalence among diarrheic children in Almadinah.

## Materials & Methods

### Sampling sites and patients

Stool samples were collected from several hospitals: Almadinah National Hospital, Maternity and Children Hospital, Mouwasat Hospital and Ohoud Hospital between January and August, 2011. A total of 889 stool samples were collected at Almadinah Hospitals. The children aged 1-12 years, 380 (42.3%) were females and 509 were males (57.7%).

**Stool samples examination** Stool samples were first examined macroscopically to ensure that they do not have gross parasitic stages (adult worms, larvae and/or

segments of tapeworms), then, microscopic examination was done by wet mount preparations, followed by the formol-ethyl acetate concentration method [8]. Finally, detection of *Giardia lamblia* copro-antigen in stool was done using the CORIS *Giardia*-Strip test.

### Detection of *Giardia* copro-antigen [9]

CORIS *Giardia*-Strip (CORIS Bioconcept, Gembloux, Belgium) is a ready-to-use test that is based on the use of a homogeneous membrane system technology with colloidal gold. The faecal sample must be diluted in the dilution buffer that is supplied with the test. A nitrocellulose membrane is sensitized with antibody to *Giardia lamblia* cysts. The test's specificity is ensured by antibody specific to the *Giardia lamblia* cyst's membrane antigens that is conjugated to the colloidal gold. This conjugate is insolubilized on a polyester membrane.

When the strip is dipped into the faecal suspension, the solubilised conjugate migrates with the sample by passive diffusion and the conjugate and sample material come into contact with the anti-*Giardia* antibody adsorbed onto the nitrocellulose. If the sample contains *Giardia lamblia* cysts the conjugate-cyst complex remains bound to the anti-*Giardia* reagent. The result – in the form of a red line that forms on the strip – is visible within fifteen minutes. The solution continues to migrate to encounter a second reagent that binds the surplus conjugate, thereby, producing a second dark red line. The test was done according to manufacturer instructions as follows:

1. 0.5mL or 15 drops of the dilution buffer solution were added to each tube.
2. The inoculating loop containing the stool sample was dipped into the tube. The dilution ratio was 2% w/v and at most 6% w/v, which equals the contents of one and three 10  $\mu$ L loops, respectively. It depended on the consistency of the stool sample: if the stool sample was solid, fewer samples was used (one loop), if the sample was liquid, more was used (three loops).
3. Stirring was done to homogenize the solution and it was left to stand for 1-2 minutes.
4. The inoculating loop was discarded and the sensitized strip was immersed in the direction indicated by the arrow.
5. The test was left to react for 15 minutes. Results were read on wet strips after 15 minutes incubation.
6. After 15 minutes, the results of the CORIS *Giardia*-Strip were read by comparing the red control band with the reaction site on the strip. If no band was visible at the reaction site the test was judged negative. If a band was visible it was judged positive.

The results were statistically analyzed and statistical significance was calculated by the Fisher test, Chi-square test and the Student's *t*-test, as applicable. Differences were to be considered statistically significant when  $P < 0.05$ .

**Results**

A total of 889 stool samples were collected at during the period from January to August 2011, from children aged 1-12 years. Of them, 380 (42.3%) were females, and 509 were males (57.7%), Table (1) shows the spatial prevalence of samples by gender throughout the study period.

Table 1. Spatial prevalence of the study cases (numbers and percentages) by gender.

Months	Numbers of Samples			Percentages	
	Male	Female	To	Male	Female
January	57	44	101	56.4%	43.6%
February	67	38	105	63.8%	36.2%
March	47	43	90	52.2%	47.8%
April	66	37	103	64.1%	35.9%
May	89	60	149	59.7%	40.3%
June	63	52	115	54.8%	45.2%
July	62	52	114	54.4 %	45.6 %
August	58	54	112	51.8 %	48.2 %
Total	509	380	889	57.3 %	42.7 %

As shown in Table 2 and Figure 1, the highest rate of positive cases was recorded in February (5.71%), while the lowest cases were recorded in July (less than 1% of total cases). No positive cases were detected in June and August. It is also noted that in January only males were positive, while in July a single female was found to be *Giardia* positive.

Table 2. Spatial prevalence of *Giardia* positive stool samples by gender.

Months	Numbers of samples	Numbers of positive samples <sup>1</sup>			Percentage
		Male	Female	Total	
January	101	3	-	3	2.97 %
February	105	3	3	6	5.71%
March	90	1	1	2	2.22 %
April	103	1	1	2	1.94 %
May	149	1	2	3	2.01 %
June	115	-	-	-	0 %
July	114	-	1	1	0.88 %
August	112	-	-	-	0 %
Total	889	9	8	17	1.91 %

<sup>1</sup> Total number of positive cases by the copro-antigen detection test

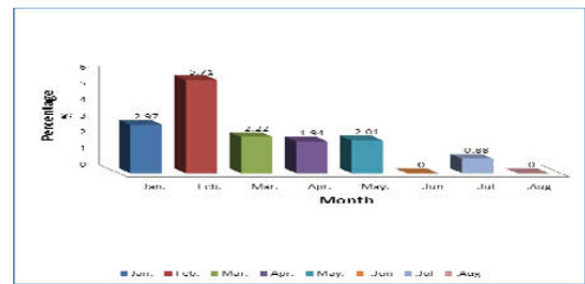


Fig. 1. Spatial prevalence of *Giardia* positive stool samples.

Among the cases' age groups, the lowest positivity rate was detected in children 1-2 years old. In contrast, the highest percentage of positive cases were detected in children aging 6-8 years of age (41.2%), followed by those between 4 and 6 years of age (29.4%). This is depicted in Table 3.

Table 3. Numbers and percentages of *Giardia* positive cases by age groups

Age group	Number of patients	Number of positive cases <sup>1</sup>	Percentages
1-2	246	1	0.4 %
2-4	165	2	1.2 %
4-6	189	5	2.6 %
6-8	120	7	5.8 %
8-12	169	2	1.2 %
Total	889	17	1.9%

<sup>1</sup> Total number of positive cases by the copro-antigen detection test

Three methods were used to detect *Giardia* in this study; direct microscopic examination, concentration technique and detection of *Giardia* copro-antigen.

Comparing the three methods and techniques, Figure 2 shows that the copro-antigen detection technique gave the best results and highest number of positive samples (n=17), followed by the concentration technique (n=14), then the direct examination (n=11). However, the differences in positivity rates were not found to be statistically significant (P > 0.05). It must be noted that no other parasites but *Giardia* (Figure 3) were detected in the study subjects.

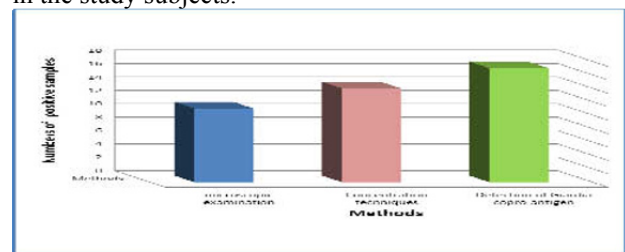


Fig. 2. Comparison between the three diagnostic methods for detection of *Giardia*.

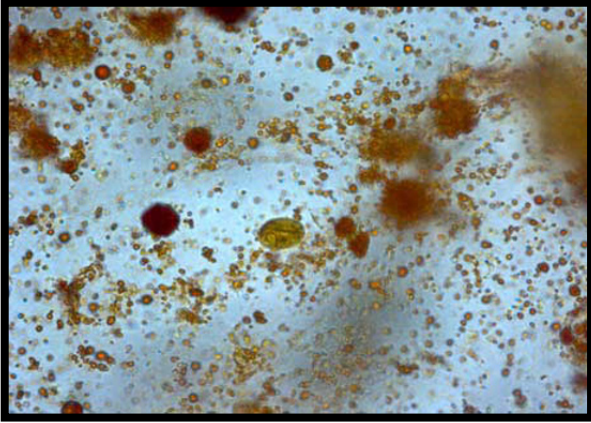


Fig. 3. Cyst of *Giardia lamblia* X100 (as pointed by arrows).

Finally, the method of *Giardia* copro-antigen test detected 17 infected cases, compared to the control strip, as seen in figures 4 and 5.



Fig 4. Positive *Giardia* antigen test band.



Fig. 5. Control band.

## Discussion

Giardiasis is a worldwide infection that is detected in all age groups although it is encountered more frequently in children. The higher prevalence in children indicates some degree of acquired resistance to infection in adults [10, 11]. Human and waterborne sources of infections are reported globally [12]. Diagnosis largely depends on direct stool sample examination, however, rapid copro-antigen detection kits and molecular techniques are being increasingly used [6, 13].

In the current study, the prevalence of giardiasis was higher in male children (52.9%) than in females (47.1%), which is in agreement with the other studies [14, 15]. This is probably due to the higher activity of male children and more contact with environment outdoors, compared to females.

The occurrence and prevalence of giardiasis varied among the ages. In the present work, it was observed that 6-8 years group was mostly infected with giardiasis, and this was closely followed by 4 - 6 years age group. This may be explained by the increasing activity of these children, being at school and playgrounds more than younger children, and also, lacking the personal hygiene than older children. This finding is in accordance with previous studies that reported that the highest risk was seen in the young children, with a decreasing risk in older children and adults [8, 16, 17].

In contrast, the lower prevalence observed in the 1 to 2 years group may be explained by the fact that the community has a culture of prolonged breast feeding of children: a 5-fold protective effect against giardiasis thanks to exclusive breastfeeding was demonstrated compared with no breast-feeding among infants 0-18 months old [18]. A hospital based surveillance study reported that breast-feeding was protective against *Giardia* infections for infants up to 6 months of age [19]. This protection, however, may be independent of the role of mother's milk protective antibodies. An in vitro study has shown that certain components of non-immune milk are capable of destroying *Giardia* trophozoites [20, 21, 22].

As for the seasonal incidence observed in this study, the finding that the highest degree of positivity was in February, while none were recorded in July and August can be either a coincidence or due to the habits of families and school timings in these periods. Perhaps most infections are acquired in the school settings, hence, with the start of the summer holidays and the presence of Ramadan in August the incidence decreased, as most eat at home then. On the contrary, it may also be a possibility that in February, the high

incidence may be due to the presence of the mid-term holiday and the habits of families to go out more, eat out or travel, all these may be factors exposing children more to such oral infections. It is noteworthy that hospital records showed that only 6 of the infected children came back for check up and stool analysis and were found parasite free after treatment.

As for diagnosis, it was decided not to rely only on direct microscopic examination of stool samples as it only detects 50 to 70% of infected patients, due to the intermittent shedding of parasite cysts and trophozoites [23]. Moreover, it is time consuming and sensitivity can be lower in chronic giardiasis [23, 24].

In this study, microscopic examination revealed that 11 (1.23%) children were infected with *G. lamblia*. Only *Giardia* cysts were identified in all positive samples.

It was decided to use a concentration method in this study as it can increase the sensitivity of ova or cyst detection by 10-12% [25]. Actually, the concentration method revealed that 14 (1.57%) were infected with *G. lamblia*; 3 more cases than direct wet mount.

Moreover, the copro-antigen CORIS rapid dipstick test was also used for diagnosis. This test detected 17 positive cases; 3 more than those diagnosed by the concentration method. All the microscopy positive cases were among those detected by the rapid dipstick test. The superior efficacy of modern dipstick copro-antigen assays is indeed stated in several previous works [26, 27, 28].

In conclusion, to the best of our knowledge, no recent studies have been done in Almadinah, documenting the incidence or prevalence of giardiasis among children. According to results of this study, giardiasis does not appear to be of the leading causes of diarrhoea in children in Almadinah, especially among toddlers. Therefore, stool examination for viral and bacterial pathogens is recommended to determine the cause of the diarrhoea in the remaining diarrheic children. As for the routine diagnosis of giardiasis, microscopic examination remains the key method. Concentration methods are to be used whenever possible. Moreover, the use of sensitive and specific copro-antigen techniques is highly advocated, especially if there is a lack of expert microscopists and for rapid screening purposes.

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