

Prevalence of *Entamoeba histolytica/Entamoeba dispar* and *Giardia lamblia* infections among children in Sebha and Mourzak cities, Libya.

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Abstract Intestinal parasitic infection is still a major public health problem in the world. The most of these parasites are distributed in the tropical and sub tropical areas. The study was conducted on the prevalence of Amoebiasis and Giardiasis among children in Sebha and Mourzak cities. A total of 156 fresh stool samples (86 males and 70 females) were collected and examined from January to December, 2016. The ages of these children ranged from one month to 14 years. *P*-values were calculated using the Chi-square test, and were considered to be statistically significant when *P* less than 0.05. The study resulted that the overall prevalence rate of *Entamoeba histolytica/dispar* and *Giardia lamblia* infections was 16.0%. A significant difference was detected between the prevalence of *E. histolytica/dispar* (12.8%) and *G. lamblia* (3.2%) ($p=0.004$). The study also found there was no significant difference ($p=0.606$) between the prevalence of infection in Sebha (18.7%) and Mourzak (12.3%). The results of stool sample revealed that the general rate of these two parasitic infections was insignificantly ($p=0.401$) higher in males (18.6%) than in females (12.9%). According to the age group of the children, the highest prevalence of infection was 22.2% and found in the group aged between 6-8 years, with no significant relationship between the rate of infection and ages ($p=0.807$). Based on the seasonal variations, the highest prevalence rates were recorded in March (37.5%) and the lowest was 4.8% in May, with significant differences between the prevalence rate in different months ($p=0.027$).

Keywords: Entamoeba, Giardia, Mourzak, Prevalence, Sebha.

دراسة معدل انتشار طفيل الزحار الأميبي *Entamoeba histolytica/dispar* وطفيل الجيارديا لامبليا *Giardia lamblia* بين الأطفال في مدينتي سبها و مرزق، ليبيا

الجيارديا لامبليا *Giardia lamblia* بين الأطفال في مدينتي سبها و مرزق، ليبيا

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المخلص تعتبر العدوى الطفيلية المعوية من أحد المشاكل الصحية في مختلف دول العالم، ولا سيما في المناطق المدارية وشبه المدارية. أجريت هذه الدراسة على معدل انتشار طفيل الزحار الأميبي *Entamoeba histolytica/dispar* وطفيل الجيارديا لامبليا *Giardia lamblia* بين الأطفال في مدينتي سبها و مرزق. من خلال هذه الدراسة تم تجميع 156 عينة براز (86 ذكور و 70 إناث) وتم فحصها خلال الفترة من شهر يناير حتى شهر ديسمبر 2016. تراوحت أعمار هؤلاء الأطفال من عمر شهر حتى 14 سنة، وتم حساب قيم *P* باستخدام اختبار مربع كاي، واعتبرت قيمة *P* أقل من 0.05 ذات دلالة إحصائية. أسفرت الدراسة عن أن معدل الانتشار الكلي للإصابة بالعدوى بهذين الطفيليين كان 16.0%. كما أظهرت النتائج عن وجود فرق معنوي ($p = 0004$) بين نسبة انتشار طفيل *E. histolytica/dispar* (12.8%) و نسبة انتشار طفيل *G. lamblia* (3.2%). كما بينت النتائج عدم وجود أي فرق معنوي ($p = 0.606$) في نسبة انتشار الإصابة بين مدينتي سبها (18.7%) و مرزق (12.3%). كما تشير الدراسة إلى أن معدل انتشار الإصابة في الذكور (18.6%) كان أعلى من الإناث (12.9%) مع عدم وجود فرق إحصائي بينهما ($p=0.401$). أيضا سجلت الدراسة أن أعلى معدل إصابة في الأطفال كان في الفئة العمرية من 6 إلى 8 سنوات وبنسبة انتشار 22.2%، مع عدم وجود فرق إحصائي بين نسب انتشار الإصابة والفئات العمرية ($p=0.807$). واستنادا إلى التغيرات الموسمية، فقد سجل أعلى معدل انتشار للإصابة في شهر مارس (37.5%) وأدنى نسبة كانت 4.8% في شهر مايو، مع وجود فروق معنوية بين معدل انتشار الإصابة وأشهر السنة ($p = 0.027$).

الكلمات المفتاحية: الزحار، الجيارديا، مرزق، الإصابة، سبها.

Introduction

Intestinal parasitic infection is still a major public health problem in the world. World Health Organization (WHO) estimates that 3.5 billion people worldwide are affected by these parasites,

with a morbidity of 450 millions [1]. The most of these parasites are distributed in the tropical and sub tropical areas. The prevalence of these infections are higher in children [2], and in

patients with Acquired Immunodeficiency Syndrome (AIDS) [3-4]. *Entamoeba histolytica* and *Giardia lamblia* are the most important protozoan parasites in the world [5]. They have similar symptoms and life cycles [6], and responsible for causing diarrhea and deaths in infected people [7]. Globally, there are more than 500 million cases for *E. histolytica/dispar* and 200 million cases for *Giardia lamblia* [8], and the majority of these infections are children. Amoebiasis and Giardiasis are two parasitic diseases caused by *Entamoeba histolytica* and *Giardia lamblia*, respectively. The first is responsible for causing 40,000 to 110,000 deaths every year [10] and considered to be the third most common parasitic disease cause of death after Malaria and Schistosomiasis [9]. Several studies have been conducted in various parts of Libya and reported different values of the prevalence of intestinal parasites among children [11-26]. The most common parasites were found in Benghazi are *E. histolytica* (3.9%) and *G. lamblia* (6.2%) [11]. These two parasites were also detected in Derna district by Sadaga and Kassem [12], with infection rates of 6.6% and 12.7% for *E. histolytica* and *G. lamblia*, respectively. Alkilani et al. in Nalout was found that children were more infected than adults, with infection rates of 21% and 2.5% for *E. histolytica* and *G. lamblia* respectively [13]. Two studies were conducted in Sebha in 2017 [21,25] reported moderate prevalence of Amoebiasis and Giardiasis with infection rates of 3.1%, 4.4% for *E. histolytica/dispar* and 1.5, 1.4% for *G. lamblia*, respectively. ESalem et al., [19] also found that the prevalence of *E. histolytica/dispar* infection in schoolchildren in Sebha was 4.0%. The aim of the presents study was to determine the prevalence of Amoebiasis and Giardiasis among children in Sebha and Mourzak cities.

Materials and Methods:

1- Study area and populations: The study was conducted on the prevalence of Amoebiasis and Giardiasis among children in Sebha and Mourzak cities from January to December, 2016. Sebha is located in the South-east of Libya between 26, 28 North, and 14,16 East, and Mourzak is situated about 130 km in the south of Sebha between 25, 55 North, and 13, 55 East. In this cities, the temperature changes rapidly from the scorching-hot days (45 °C) to the cooler nights (4 °C).

A total of 156 fresh stool samples (86 males and 70 females) were collected for the study from two cities: Sebha (91 sample) and Mourzak (65 sample). The sample population consisted of 86 (55.1%) males and 70 (44.9%) females aged between one month to 14 years, and divided into 5 age groups.

2- Data and sampling collection: Fresh stools were collected in labeled clean plastic containers. Prior to collection, sampling date and the personal information including name, location, sex and age were recorded for each child. Each sample was then subjected for macroscopic examination (consistency, colour, and occurrence of blood and mucus) and all the observations were recorded.

3- Examination of the samples: Fecal specimens were examined on the same day of collection by

direct wet smears by using both normal saline solution and Lugol's iodine [27]. The samples were examined in two laboratories: Al-Yamama (in Sebha) and Nesmat-Amal (in Mourzak). The identification of cysts and/or trophozoites of *E. histolytica/dispar* and *G. lamblia* found in the stools was done using keys and descriptions [28].

4- Statistical analysis: Collected data were analyzed using Statistical Package for Social Sciences (SPSS, version 20, Inc, Chicago, IL, USA). P-values were calculated using the Chi-square test, and were considered to be statistically significant when P less than 0.05 ($p < 0.05$).

Results: One hundred and fifty six stool samples from children were examined From January to December, 2016. The ages of these children ranged from one month to 14 years, and the number of males (55.1%, $n=86$) was slightly higher than females (44.9%, $n=70$). The study resulted that the overall prevalence rate of *E. histolytica/dispar* and *G. lamblia* infections was 16.0% (25 out of 156), with prevalent rate of 12.8% (20 out of 156) for *E. histolytica/dispar* and 3.2% (5 out of 156) for *G. lamblia* (Figure 1). A significant difference was detected between the prevalence of *E. histolytica/dispar* and *G. lamblia* ($p=0.004$). The prevalence of *E. histolytica/dispar* ranged from 7.7% in Mourzak to 16.5% in Sebha, whereas, *G. lamblia* prevalence varied from 2.2% in Sebha to 4.6% in Mourzak, with no significant differences were found between the prevalence of infection in Sebha (18.7%, 17 out of 91) and Mourzak (12.3%, 8 out of 65) ($p=0.606$). No mixed infections were found with these two parasites.

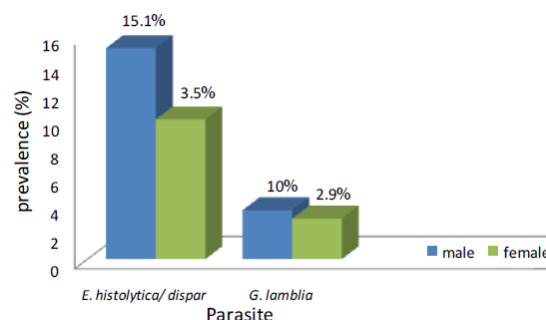


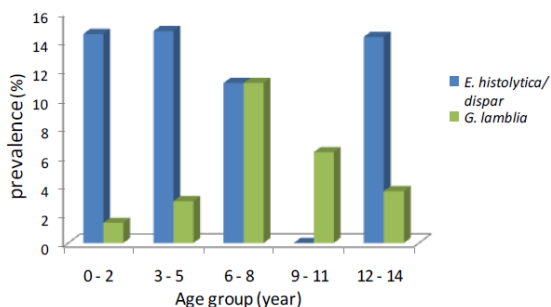
Fig. 1: The distribution of *E. histolytica/dispar* and *G. lamblia* infections among children according to gender.

As indicated in Table (1), the results of stool sample revealed that the general rate of these two parasitic infections was insignificantly higher in males (18.6%) than that in females (12.9%). No significant associations were detected between sex and the prevalence of *E. histolytica/dispar* or the prevalence of *G. lamblia* ($p=0.401$).

Table 1: Prevalence of *E. histolytica/dispar* and *G. lamblia* according to gender.

Sex	NO.OF examined	Parasita	<i>E.histolytica /dispar</i>	<i>G.lamblia</i>	Total
Male	86	NO.infected	13	3	16
		Prevalence (%)	15.1	3.5	18.6
Female	70	NO.infected	7	2	9
		Prevalence (%)	10.0	2.9	12.9
Total	156	NO.infected	20	5	25
		Prevalence (%)	12.8	3.2	16.0

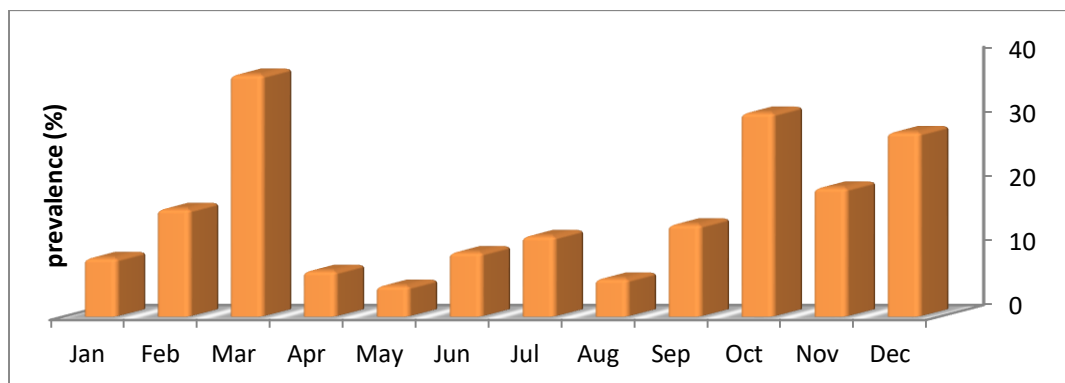
According to the age group of the children, the results found no significant relation between age group and infectivity rate of *E. histolytica/dispar* and *G. lamblia* at ($p=0.807$). These two parasites were more prevalent in older age group (6-8 years) with prevalent rate of 22.2% (Figure 2), while the lowest infection rate was 6.3% in the 9-11 years age group (Table 2).

**Fig. 2:** The distribution of *E. histolytica/dispar* and *G. lamblia* infections among children according to age group.**Table 2: Prevalence of *E. histolytica/dispar* and *G. lamblia* according to age group.**

The result	Age group (years)					Total
	0-2	3-5	6-8	9-11	12-14	
<i>E.histolytica/dispar</i>	10	5	1	0	4	20
	14.5%	14.7%	11.1%	0.0%	14.3%	12.8%
<i>G.lamblia</i>	1	1	1	1	1	5
	1.4%	2.9%	11.1%	6.3%	3.6%	3.2%
NO.examined	69	34	9	16	28	156
Total NO.infected	11	6	2	1	5	25
Prevalence (%)	15.9	17.6	22.2	6.3	17.9	16.0

Based on the seasonal variations, the highest prevalence rates were recorded in March (37.5%) and October (31.6%), and the lowest was 4.8% in May (Figure 3). Furthermore, all the infected samples with *G. lamblia* (5 cases) were in the months from October to December (Table 3). The statistical significant differences between the prevalence in different months were observed ($p=0.027$). The mean prevalence rate of Winter months was 21.2% and higher compared the mean of Summer months (8.1%).

Discussion: Intestinal parasitic infections are still a major public health concern in the world, especially in tropical and subtropical countries. In Libya, various studies were conducted in different cities to investigate the prevalence of the most common intestinal protozoan parasites among children. The aim of the present study to determine the prevalence rate of *E. histolytica/dispar* and *G. lamblia* infection among children

**Fig. 3:** Seasonal variations of the prevalence of protozoan parasitic infection**Table 3: The distribution of intestinal parasites in infected people stratified by month.**

Months	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
NO.examined	11	12	8	14	21	10	8	17	7	19	15	14	156
NO.infected	1	2	3	1	1	1	1	1	1	6	3	4	25
Prevalence (%)	9.1	16.7	37.5	7.1	4.8	10.0	12.5	5.9	14.3	31.6	20.0	28.6	16.0

who visited Al-Yamama and Nesmat-Amal laboratories in Sebha and Mourzak, respectively. A total of 156 stool specimens were examined during the period from January to December, 2016.

This study Concentrated on only two parasites namely: *E. histolytica/dispar* and *G. lamblia*

because the infections with these parasites are responsible for causing diarrhea and deaths in infected people, especially in children [7,22]. Furthermore, they were the most common intestinal pathogenic protozoa reported in the most publications and distributed in children under the age of 15 years [30]. *E. histolytica* is a

pathogenic parasite, whereas, *E. dispar* is a non-pathogenic parasite. In the current study, using the two parasites together as *E. histolytica/ dispar* because they are microscopically indistinguishable. Two studies were done in Saudi Arabia [28] and Italy [29] in 2007 and 2011, respectively. The former found the presence of *E. histolytica/* or *E. dispar* in 65% of the samples by using the microscope and found the *E. histolytica* in 2.6% only by using the second generation *E. histolytica*-specific EIAs. Whereas, the latter detected *E. histolytica/* or *E. dispar* microscopically in 33 patients, and in only 11 patients of these found *E. histolytica* molecular techniques. The results of this study indicated that the overall prevalence of intestinal protozoan infection was 16.0% which is similar to the previous studies carried in Ethiopia (16.6%) [30], Al-Khoms city (15.4%) [20], and in Sebha (14.8%) [23] and 14.9% [25]. This finding was higher than other reports in Libya in Benghazi (12.9%)[11] and Zawia (7.4%)[28]. In contrast, the result was lower than studies done in Derna by Sadaga and Kassem (31.0%)[12]; and in Nalout by Alkilani et al., (29.6%)[13]. Prevalence of *E. histolytica/dispar* was significantly higher than *G. lamblia* which may be due to the cyst of the former may be more active and more resistant to the environment conditions than the cyst of the latter. This result was in agreement with the previous results obtained in Sirt by Kassem et al., [14]; Zliten by Ali et al., [15]; Iraq by Al Saqur et al., [31] and Egypt by El-Masry et al., [32]. However, there was no significant differences were noted between the infection prevalence with each parasite in Sebha and Mourzak. The absence of statistical differences between the prevalence of infection in Sebha and Mourzak may attributed to that both cities are exposed to the same weather conditions. Our investigators found insignificantly higher prevalence rates of *E. histolytica/dispar* and *G. lamblia* among males (18.6%) than females (12.9%). This finding agrees with results by Elsaid et al., (11.3% in males vs 10.1% in females)[26] and ESalem et al., (6.0% in males vs 3.0% in females)[19]. The higher percentage of infection in males may referred to many reasons: males are more active and more free than females, the social controls or restrictions imposed on females, and also because of the increased phenomenon of eating fast food outside the homes. The results of this study found no statistical significant differences between the rate of intestinal parasitic infection and age groups ($p=807$). The highest prevalence (22.2%) was recorded in children aged 12-14 years old (Table 2). This finding agrees with results by Alsirieti et al., [18] who reported the high prevalence was in the age group of 2-10 years old. In contrary, Gelaw et al. [33] and Muhajir et al., [34] recorded the highest rates were in the age group of 10-12 and 11-20 years, respectively. Whereas Kassem et al., [14] reported that the majority of infections with *E. histolytica/dispar* and *G. lamblia* infections occurred in children aged 1-4. The result showed seasonal variations in the prevalence of *E. histolytica/dispar* and *G. lamblia* infections

throughout the year. The highest prevalence were recorded in March (37.5%) and October (31.6%), and the lowest was in May (4.8%). (Table 3). It is also noted that the mean prevalence rate of Winter months was 21.2% and was higher compared the mean of Summer months (8.1%). This finding may due to the high temperature in Summer months (over 45 °C) may affect on the cyst viability of protozoan parasites.

Conclusion: This study showed higher prevalence of *E. histolytica/dispar* than *G. lamblia* among children in Sebha and Mourzak cities, Libya. In order to obtain accurate results in future studies on the prevalence of *E. histolytica/dispar* study, the need for the use of modern sensitive techniques such as the multiplex real-time PCR and ELISA test is very important to differentiate between these two species.

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