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A survey of the prevalence of intestinal protozoa in Sebha city, Libya

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Abstract The study was conducted on the prevalence of intestinal protozoan parasites in Sebha city, Libya. Collection of 1,526 stool samples were undertaken from January to December 2014. All samples were examined in the Sebha Central Laboratory. The objective of the study was to measure the prevalence infection of intestinal protozoan in Sebha city. The Chi-square test was used to measure the statistical significant differences at level $p<0.05$. The results of the study showed that, 14.8% of the examined samples were infected with different species of protozoan parasites. *Blastocystis hominis* was the most common parasite detected in this study, with 9.8% of infection rate, followed by *Entamoeba histolytica/Entamoeba dispar* (3.1%), and then *Giardia lamblia* (1.5%), *Entamoeba coli* (0.3%), *Trichomonas hominis* (0.13%), and finally *Entamoeba hartmani* (0.07%). The prevalence of infection in females (15.8%) was insignificantly ($p=0.357$) higher than in males (13.9%). The highest prevalence (19.5%) was found in the group aged between 10 to 19 years, with a significant relationship between the intestinal protozoan infection and ages ($p=0.004$). The results revealed that the highest rate of infection was in February (21.9%), followed by 21.1% in January, with no significant differences in the prevalence of infection between the different months ($p=0.706$).
Keywords: Sebha; prevalence; intestinal protozoa.

دراسة نسبة انتشار الأوليات الطفيلية المعوية في مدينة سبها– ليبيا

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الملخص أجريت هذه الدراسة على معدل انتشار الأوليات الطفيلية المعوية التي تصيب الأمعاء في مدينة سبها، ليبيا. تم تجميع 1526 عينة براز خلال الفترة من يناير الى ديسمبر 2014، وقد تمت عملية فحص العينات في المختبر المركزي بسبها. تهدف هذه الدراسة الى قياس معدل انتشار الاصابة بالأوليات الطفيلية في مدينة سبها، وتمت دراسة العلاقة بين نسبة الاصابة مع كلا من الجنس، الفئات العمرية، وأشهر السنة باستخدام اختبار مربع كاي Chi-square test واعتبرت النسبة أقل من 5% ذات دلالة احصائية. أظهرت النتائج أن حوالي 14.8% من العينات المفحوصة كانت مصابة [انواع مختلفة من الأوليات الطفيلية. ويعتبر طفيل البلاستوسيستس هومينس *Blastocystis hominis* من أكثر الطفيليات شيوعا في هذه الدراسة وبنسبة انتشار 9.8%، يليه طفيل الزحار الأميبي *Entamoeba histolytica/Entamoeba dispar* وبنسبة 3.1%، ثم طفيل الجيارديا لامبليا *Giardia lamblia* بمعدل 1.5%. بقية الطفيليات هي طفيل الأميبا القولونية *Entamoeba coli* (0.3%)، طفيل المشعرة البشرية *Trichomonas hominis* (0.13%)، وطفيل الاميبا الهارتمانية *Entamoeba hartmani* (0.07%). تشير الدراسة الى أن معدل انتشار الاصابة في الإناث (15.8%) كان أعلى من الذكور (13.9%) مع عدم وجود فرق احصائي بينهما ($p=0.357$). أيضا سجلت الدراسة أعلى معدل اصابة كان في الفئة العمرية من 10 الى 19 سنة وبنسبة انتشار 19.5%، مع وجود فرق احصائي بين نسب انتشار الاصابة والفئات العمرية ($p=0.004$). كما أوضحت هذه النتائج أن أعلى معدل لانتشار الاصابة كان في شهر نوفمبر (21.9%)، يليه شهر يناير (21.1%)، مع عدم وجود فرق احصائي بين نسبة انتشار الاصابة وأشهر السنة المختلفة ($p=0.706$).
الكلمات المفتاحية: سبها، انتشار، الأوليات الطفيلية المعوية.

Introduction:

Intestinal parasitic infection is endemic throughout the world, especially in the developing country like Libya. In the year 2000, WHO reported that The effect and morbidity of the protozoan parasites estimated 3.5 billion and 450 million persons respectively (WHO, 2000). Human intestinal parasites are responsible for the most gastrointestinal disorders found both in developed and developing countries. Intestinal signs caused by these parasites are include abdominal pain, acute or chronic diarrhea, weight loss, nausea, vomiting and anemia (Masucci et al., 2014). *E. histolytica* and *G. lamblia* are the most two protozoa associated with diarrhea, and they are causative agents of amebiasis and giardiasis, respectively (Fletcher et al., 2012). The transmission of the most protozoan parasites can be occurred by the faecal-oral route, and the most affected groups are children and young adults. Moreover, causes of parasitic infections are

increasing by many factors such as poor socioeconomic status, poor hygienic environment, climate, overcrowding of people (Niyyati et al, 2009) and immigration of workers from the neighbouring countries (Nowara and Nair, 2005). There are many studies on the intestinal parasitic infections in Libya. Some of these studies were confined on school children, and others were on both children and adults. In Benghazi, the first study was conducted by Dar et al (1979) on school children, followed by EL-Boulaqi et al (1980) for the prevalence of intestinal parasites in primary school children. Another study was done by EL-Buni and Khan (1998) on the intestinal protozoan infections. Another four studies were conducted in Benghazi, two studies on the intestinal protozoan infections by El-Ammari & Nair (2003) and by Nowara & Nair (2005), whereas, the others were on the intestinal protozoan and helminth parasites by El-Ammari et al., (2004) and Kubti et al., (2011). In Tripoli, about five studies were done, the first was in 2006 by Ben-Rashed et al., and the second by Ben-Musa (2007). Two of these studies were in 2007 by Ben-Musa et al.; and the last study was by Rahouma et al.; (2011). Three studies were studied in Sirt by Alsirieti et al., (2006); Abdel-Magied and Elahwel (2006); and Kassem et al., (2007). In Derna, Sadaga and Kassem (2007) studied the prevalence of intestinal parasites in school children. Elsaid et al.(2014); El-Ammari & Nair (2015); and Mergani et al.,(2014) studied the prevalence of protozoan parasites in Zawia; Al-Khoms and Brack, respectively. Alkilani et al.(2008) and Saad et al.(2009) studied the incidence of intestinal parasites in Nalout and Wadi Al-Shati region, respectively. Ali et al.(2005) conducted a study on the prevalence of intestinal parasites in children in Zliten. In Sebha, two studies were conducted by ESalem et al., (2017) and Ibrahim and Salem (2017). The objective of this study is to determine the prevalence of intestinal protozoa infection in Sebha city- Libya.

Materials and Methods:

Study area: The study was conducted on the prevalence of of intestinal protozoan parasites in Sebha city, Libya. Sebha is situated in the South-east of Libya between 26, 28 North, and 14,16 East. In this city, the temperature changes rapidly from the scorching-hot days (45 ◦ C) to the cooler nights (4 ◦ C).

Study populations: A total of 1526 fresh stool samples (796 males and 730 females) were collected for the study during the period from January to December, 2014. The ages of these cases ranged between 0 - 84 years, and divided into 7 age groups.

Data and sampling collection: Fresh stools were collected in clean, numbered plastic containers. Collection date and the personal information including name, sex and age were recorded for each sample. The macroscopic examination of each sample (consistency, coulor, and occurrence of blood and mucus) was checked. All the observations were recorded.

Examination of the samples: Fecal specimens were examined on the same day of collection by direct wet smears by both normal saline solution and Lugol's iodine as described by Markell et al.(1999). Stool samples were not examined for *Cryptosporidium* spp. The identification of parasites cysts and/or trophozoites found in the stools was done using keys and descriptions (Jeffrey and Leach, 1991).

Statistical analysis: Epi Info 2000 software was employed. *P*-values were calculated using the Chi-square test. $p<0.05$ was considered to be statistically significant.

Results: From January 2014 to December in the same year, 1526 faecal samples from children and adults attending to Sebha Central Laboratory were examined for intestinal protozoan parasites. The highest number of the samples was collected in months of April and May (216 and 201 respectively), whereas, the lowest number (38 sample) was in January. The ages of these people ranged from 0 to 84 years, and slightly more than half of samples (52.2%, n=796) was males.

The results revealed that 14.8% (226/1526) of the total samples were positive with protozoan parasites. *B. hominis* was the most common parasite detected in this study, with prevalent rate of 9.8%, followed by *E. histolytica/E. dispar* (3.1%), *G. lamblia* (1.5%), *E. coli* (0.3%), *T. hominis* (0.13%), and finally *E. hartmani* (0.07%). However, a significant association (p=0.0001) was observed between the prevalences of infection of these protozoan parasites.

The results in Table 1 showed that the prevalence of infection in males was 13.9% (111/796), and a slight lower than in females (15.8%; 115/730), with no significant differences in the prevalence of infection between males and females (p=0.357). The distribution of the positive samples showed in figure 1.

Table 1:Prevalencwof Intestinal protozoa according to sex.

Sex		The result		
		positive	negative	Total
male	No.samples	111	685	796
	Prevalence%	13.9%	86.1%	100%
female	No.samples	115	615	730
	Prevalence%	15.8%	84.2%	100%
Total	No.samples	226	1300	1526
	Prevalence%	14.8	85.2	100%

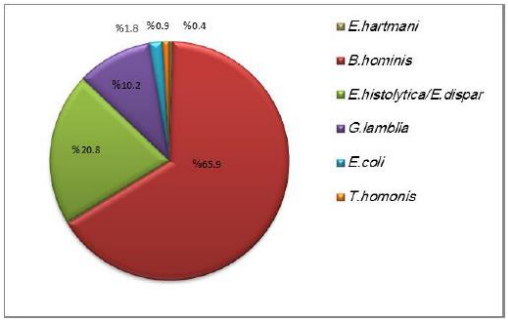

Figure 1: The distribution of the positive samples with the protozoan parasites.

According to the age group, the results showed that the highest prevalence of protozoan infection was 19.5% (40/205), and it found in the group aged between 10 to 19 years, and the lowest

percentage was found in the group aged between 30 to 39 years with 9.1% (17/186). The results indicated in table 2 showed that there was a significant relation between the prevalence of intestinal protozoa and the age (p=0.004). The prevalence of protozoan infections had a higher rates in the months of February and January during the study year. The highest monthly rate was in February (21.9%, 16/73), followed by 21.1% (8/38) in January. The lowest rates were 11.8% in both March (23/195) and August (11/93), with no significant differences in the prevalence of

Table2 : :Prevalence of protozoan infections according to age group.

The result	Age group							Total
	0-9	10-19	20-29	39-30	49-40	59-50	≥60	
positive	54	40	41	17	34	24	16	226
	11.0%	19.5%	18.6	9.1%	18.3%	17.8	15.8%	14.8%
negative	438	165	180	169	152	111	85	1300
	89.0%	80.0%	81.4%	90.9%	81.7%	82.2%	84.2%	85.2%
Total	492	205	221	186	186	135	101	1526
	100%	100%	100%	100%	100%	100%	100%	100%

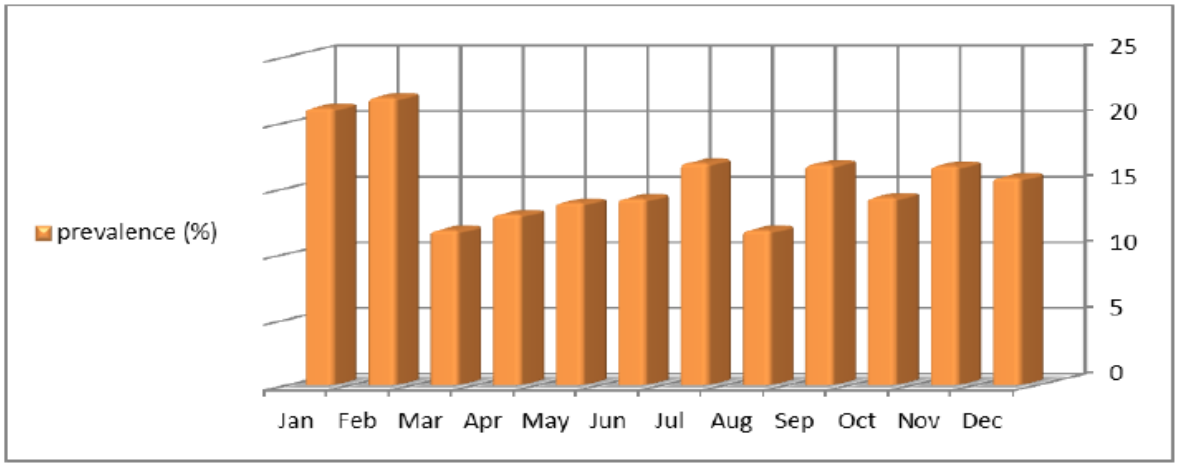

Figure 2: Prevalence of the intestinal protozoan parasites according to months.

infection between the different months (p=0.706). The infection prevalence during the study period shown in figure 2, and the distribution of intestinal parasites in infected people stratified by age and sex and by month shown in Tables 3 and 4 respectively.

Table 3: The distribution of intestinal parasites in infected people stratified by age and sex

parasite	No. Of infected samples (%)							Total		
	Age group							Total	Sex	
	0-9	10-19	20-29	30-39	40-49	50-59	≥60		Male	Female
B.hominis	30	25	26	11	24	22	11	149	70	79
	13.3%	11.1%	11.5%	4.9	10.6%	9.7	4.9	65.9%	31.0%	35.0%
E.histolytical	11	11	7	3	10	0	5	47	27	20
E.dispar	4.9%	4.9%	3.1%	1.3%	4.4%	0.0%	2.2%	20.8%	11.9%	8.8%
G.lamblia	11	3	6	3	0	0	0	23	12	11
	4.9%	4.9%	2.7%	1.3%	0.0%	0.0%	0.0%	10.2%	5.3%	4.9%
E.coli	1	1	1	0	0	1	0	4	1	3
	0.4%	0.4%	0.4%	0.0%	0.0%	0.4%	0.0%	1.8%	0.4%	1.3%
T.hominis	0	0	1	0	0	1	0	4	1	3
	0.0%	0.0%	0.4%	0.0	0.0%	0.4%	0.0%	1.8%	0.4%	1.3%
E.hortmani	1	0	0	0	0	0	0	1	0	1
	0.4%	0.0%	0.0%	0.0%	0.0%	0.0	0.0	0.4	0.0%	0.4%
Total	54	40	41	17	34	24	16	226	111	115
	23.9%	17.7%	18.1%	7.5%	15.0%	10.6%	7.1%	100.0%	49.1%	50.9%

Discussion: Intestinal protozoan parasites are major public health problems in developing countries. Their infections affect a huge number of people, and responsible for the morbidity in 450

million person in the world (WHO, 2000). The main causal agents of gastrointestinal disorders are diarrhoea, dysentery, weight loose, vomiting, abdominal pain and anaemia (Bethony et al., 2006). Very little is known and studied about the prevalence of intestinal protozoa in the south of Libya. The present study was conducted at Sebha Medical Centre over a 12-month period in 2014. The current findings indicated that the overall prevalence of intestinal protozoan infection was 14.8% which is equal to the study in Sebha

(14.9%) and Al-Khoms city (15.4%) by Ibrahim and Salem (2017) and El-Ammari and Nair (2015), respectivaley. The present result is lower in comparison to studies carried in some cities in Libya and other countries in the world. In Libya, these results were obtained by Boulaqi et al.,(1980) in Benghazi (75.6%); Sadaga and Kassem (2007) in Derna (31.0%); and Alkilani et al., (2008) in Nalout 29.6%). Whereas in other countries were conducted by Akhter et al. (1994) in Saudi Arabia (27.8%); Farag (1985) in Yemen (53%); and Mehraj et al.,(2008) in Pakistan (52%). On the other hand, the current result (14.8%) seems to be higher than other findings in Libya studied by Elsaid et al., (2014) in Zawia (7.4%) and El-Buni and Khan (1998) in Benghazi (12.9%).

Table 4:The distribution of intestinal parasites in infected people stratified by month.

Month	Protozoan parasites							The result	
	B.hominis	E.histolytica E.dispar	G.lambla	E.coli	T.hominis	E.hartmani	Total	positive	negative
January	5	2	1	0	0	0	38	8	30
	2.2%	0.9%	0.4%	0.0%	0.0%	0.0%	100.0%	21.1%	78.9%
February	14	0	1	0	0	1	73	16	57
	6.2%	0.0%	0.4%	0.0	0.0	0.4%	100.0%	21.9	78.1
March	17	5	1	0	0	0	195	23	172
	7.5%	2.2%	0.4%	0.0%	0.0%	0.0%	100.0%	11.8%	88.2%
April	16	7	3	1	1	0	216	28	188
	7.1%	3.1%	1.3%	0.4%	0.4%	0.0%	100.0%	13.0%	87.0%
May	14	9	4	1	0	0	201	28	173
	6.2%	4.0%	1.8%	0.4	0.0%	0.0%	100.0%	13.9	86.1
June	13	4	1	1	1	0	141	20	121
	5.8%	1.8%	0.4%	0.4%	0.4%	0.0%	100.0%	14.2%	85.8%
July	8	2	1	0	0	0	65	11	54
	3.5%	0.9%	0.4%	0.0%	0.0%	0.0	100.0%	16.9%	83.1%
August	10	1	0	0	0	0	93	11	82
	4.4%	0.4%	0.0%	0.0%	0.0%	0.0%	100.0%	11.8%	88.2%
September	14	2	1	0	0	0	101	17	84
	6.2%	0.9%	0.4%	0.0%	0.0%	0.0%	100.0%	16.8%	83.2%
October	7	1	2	1	0	0	77	11	66
	3.1%	0.4%	0.9%	0.4%	0.0%	0.0%	100.0%	14.3	85.7%
November	18	8	4	0	0	0	180	30	150
	8.0%	3.5%	1.8%	0.0%	0.0%	0.0%	100.0%	16.7%	83.3%
December	13	6	4	0	0	0	146	23	123
	5.8%	2.7%	1.8%	0.0%	0.0%	0.0%	100.0%	15.8%	84.2%
Total	149	47	23	4	2	1	1526	226	1300
	65.9%	29.8%	10.2%	1.8%	0.9%	0.4%	100.0%	14.8	85.2%

In this study the positive rate of intestinal protozoa in females was higher than in males. The percentages were 15.8%) & (13.9%) in females and males respectively, but it is not statistically significant (p=0.357). This finding is similar to the result by Okyay et al.,(2004) who found the prevalence in females (50.9%) was higher than in males (49.1%). On the contrary, Elsaid et al.,2014 found the positive rate was (10.1%) in females and (11.3%) in males. The high prevalence of infection in females may referred to the eating unwashed fruits and vegetables by women which may be contaminated with protozoan cysts. On the other hand, the infection rate of protozoa was similar between male and female in two studies conducted By Ngui et al.; (2011) in Malaysia and Sah et al.; (2013) in Nepal with infection rate of 18.4% vs 18.6% and 73% vs 73.3%, respectively. The present study found that there is a significant deference (p= 0.004) between the rate of intestinal parasites infection and age groups. This result agrees with studies by Gelaw et al. 2013 who found the high prevalence of intestinal protozoa in the age group of 10–12

years. This finding may be due to the activity of people in the ages between 10-19 years. As well as, Masucci et al., (2011) found that the overall prevalence 1.3% (69/5351), and the rate was increased three times in children less than 15 years of age (4.8% ; 38/797). In contrast, Dar et al, 1979; Sadaga and Kassem, 2007; and Elsaid et al., 2014 found no statistical differences between age groups (p>0.05). The most significant relations in this study between the prevalence of intestinal protozoa and the age were in the two age groups (0-9 and 30-39) in relation to the other age groups. In this study the prevalence of intestinal protozoan parasites was found significantly higher in February (21.9%) and January (21.1%), and low in August (11.8%). This finding may attributed to the increase of eating unwashed fresh salad at this time and/or the high temperature (>45 ◦ C) in Summer months may affect on the cyst of protozoan parasites.

As mentioned previously, six intestinal protozoan parasites were detected in this study namely: *B. hominis*, *E. histolytica/E. dispar*, *G. lamblia*, *E. coli*, *T. hominis*, and *E. hartmani*. *B. hominis* was the most common parasite detected in this study. This parasite has a worldwide distribution and the most commonly Parasite in the world (Aguiar et al., 2007), and still not clear whether this parasite is a pathogen or commensal (Tan, 2008; Shlim et al., 1995). In this study, The infection rate of *B. hominis* was 9.8% (149/1526). It represented 65.9% from the infected people (149/226). The level of infection by *B. hominis* was higher among

children less than nine years of age (0-9), it was 20.1% from the infected people with this parasite (30/149).The second most frequently identified intestinal Protozoa parasite detected in this study was *E. histolytica/E. dispar*. *E. histolytica* was a pathogenic protozoan parasite causing amoebiasis, and responsible for the mortality in the world. In this study, the use of two parasites together as *E. histolytica/E. dispar* because it cannot microscopically distinguish between these two parasites. A study in Italy in 2011 by Masucci et al., found that *E. histolytica/* or *E. dispar* found in 33 patients by microscopy, and in only 11 patients of these by molecular techniques. Another study in 2007 from Saudi Arabia by Al-Harthi and Jamjoom found the presence of *E. histolytica/* or *E. dispar* in 65% of the samples by microscopy and by using the second generation *E. histolytica*-specific EIAs found the *E. histolytica* in 2.6% only. The findings of this study resulted that the infection rate with this parasite was 3.1% (47/1526), and equal result was obtained in Zawia by Elsaid et al., (2014) who found 3.1% infection rate. Different percentages for this parasite were reported in Libya, 11.8% in Zliten (Ali et al., 2005); 3.0% in Tripoli (Ben-Rashed et al., 2006); 16.3% in Sirt (Abdel-Magied and Elahwel, 2006); 21% in Nalout (AlKilani et al., 2008); 6.6% in Derna (Sadaga and Kassem, 2007); 1.2% in Wadi-Alshati (Saad et al., 2009); 4.4% in Benghazi (Kubti et al., 2011); and 12.1% in Alkhoms (El-Ammari and Nair, 2015). The level of infection by *E. histolytica/E. dispar* was higher among the first two age groups (0-9 and 10-19), they were 23.4% from the infected people with this parasite (11/47). Study by Sadaga and Kassem (2007) in Derna city resulted that the prevalence of *E. histolytica/E. dispar* was significantly higher in children of less than 10 years compared with the other age groups. Ximenez et al.,(2009) is also found that *E. histolytica* mostly affect children less than 15 years of age, with a significant increase in those aged 5-9 years.The third most common species in this study was *G. lamblia*, which is also a pathogenic protozoan parasites causing giardiasis, and infects 200 million person in the world. This parasite has been recognized as one of the most significant causes of diarrhea in children and early infancy (Muhsen and Levine, 2012). The current findings indicated that the prevalence of *Giardia* infection was 1.5% (23/1526), which similar to the results in other cities:1.3% in Tripoli (Rahouma et al. 2011); 1.3% in Brack (Mergani et al., 2014); 1.2% in Zliten (Ali et al., 2005); 1.8% in Zawia (Elsaid et al., 2014); 1.8% in Wadi-Alshati (Saad et al., 2009). Furthermore, this result (1.5%) is lower in comparison to studies carried out by Ben-Rashed et al., (2006) in Tripoli (17%); Abdel-Magied and Elahwel (2006) in Sirt (28.8%); and Sadaga and Kassem(2007) in Derna (12.7%). The level of infection by *G. lamblia* was higher in children under nine years of age (0-9), it was 47.8% from the infected people with this parasite (11/23). Our results of *E. histolytica/E. dispar* and *G. lamblia* infections confirmed to those were obtained by Alsiriti et al., (2006) who indicated that the infections with these two protozoan parasites are common in the people aged between 2 to 10 years compared to other age groups. The fourth common species in this study was *E. coli*, which was detected only in four indiviuals (0.3%), three were females, and one male. The current result was low in comparison with different rates were reported in Zawia 3.8% by Elsaid et al.(2014); in Benghazi (11.4%) by Dar et al., (1979); 19.7% by EL-Boulaqi et al., (1980), and 6.24% by EL-Buni and Khan (1998).In this study, *T. hominis* was found in two persons (0.13%, 2/1526) one was male and one female, wherase, *E. hartmani* was only found in one female (0.07%; 1/1526).In conclusion, the present study showed low prevalence of intestinal protozoan infections with *E. histolytica/E. dispar* and *G. lamblia* compaired with the previous studies in Libya. Therefore, in addition to microscopic examinations, we suggest further methods like molecular techniques or *E. histolytica*-specific EIAs to distinguish between the pathogenic *E. histolytica* and the non-pathogenic *E. dispar.* Furthermore, the examination of several samples of stool per person is still the alternative way to confirm all detected results.

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