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Prevalence and seasonal variation of *Entamoeba histolytica* and *Giardia lamblia* in Kuwait

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Abstract

Background: This study was conducted to determine the prevalence of *Entamoeba histolytica* and *Giardia lamblia* in Kuwait and variations in infection rates by season. Methods: In total, 6423 samples were collected from five general hospitals and one specialised hospital in Kuwait from January 2018 to July 2019. Samples were examined using direct saline smears and Lugol's iodine staining. Samples in which parasites were not detected using wet mounts were further examined using a trichrome staining concentration technique. Results: Of the 115 positive cases of intestinal parasites (1.79%), *G. lamblia* was the most prevalent, detected in 69 samples (60%), followed by *E. histolytica* in 38 samples (33%), *Iodamoeba buetschlii* in four samples (3.5%), *Hymenolepis nana* in two samples (1.7%) and

Entamoeba coli in two samples (1.7%). The prevalence of intestinal protozoa changed according to season, with higher infection rates in spring and summer (61 infections) (57%) than in autumn and winter (46 infections) (43%). Conclusion: Low prevalence of *E. histolytica* and *G. lamblia* parasitic infection in Kuwait is an indicator of the high level of health services provided to the general population. However, there remains room for further development and improvement of strategies aimed at protecting public health.
Keywords: intestinal protozoa; *Entamoeba histolytica*; *Giardia lamblia*; parasites; parasitic infection; Kuwait.

*** Introduction**

Intestinal protozoan infection is one of the most common parasitic diseases worldwide and is a major public health issue. Infection usually occurs from the contamination of food or water with faecal matter (Dagci et al., 2008; Department of Control of Neglected Tropical Diseases, 2009). According to estimates from the World Health Organization (WHO), approximately 3.5 billion people are infected by intestinal parasites, 350 million of whom will develop disease (Mezied, Shaldoum, Al-Hindi, Mohamed, & Darwish, 2014). The two most common intestinal protozoa are *Entamoeba histolytica*, estimated to cause approximately 100 000 deaths per year from invasive amoebiasis (Carrero et al., 2020; Kantor et al., 2018), and *Giardia lamblia*, the causative agent of giardiasis, with approximately 500 000 newly reported cases per year according to the WHO (1996).

Many protozoa reside in the digestive system, and most are not pathogenic. However, under favourable conditions, some protozoa can cause serious symptoms, including diarrhoea, iron-deficiency anaemia, malnutrition and even growth retardation in children (Galgamuwa, Iddawela, & Dharmaratne, 2016; Quihui-Cota et al., 2017; Rodríguez-Morales et al., 2006). Further, *G. lamblia* is responsible for various gastrointestinal diseases and is one of the major causes of traveller's diarrhoea (Assudani, Gusani, Mehta, & Agravat, 2015; Bartelt & Sartor, 2015). *E. histolytica* and *G. lamblia* can be transmitted to humans directly via the faecal–oral route or indirectly through food or water contaminated with human or animal faeces (Al-

Mohammed, Amin, Aboulmagd, Hablus, & Zaza, 2010). However, other factors can affect transmission, including socioeconomic status (Jaran, 2016), age (Mehraj, Hatcher, Akhtar, Rafique, & Beg, 2008), education (de Almeida, Jeske, Mesemburg, Berne, & Villela, 2017; Okyay, Ertug, Gultekin, Onen, & Beser, 2004), personal hygiene (Jejaw, Zeynudin, Zemene, & Belay 2014; Mahni et al., 2016; Tandukar et al., 2013), travel and immigration (Al-Mohammed et al., 2010), and access to clean drinking water and proper sanitation (Curval et al., 2017). These factors play major roles in the prevalence and distribution of *E. histolytica* and *G. lamblia*.

Some studies have been conducted in Kuwait to investigate parasitic infections in terms of prevalence and contributory factors such as low education levels, personal hygiene habits and socioeconomic status (Al-Nakkas, Al-Mutar, Shweiki, Sharma, & Rihan, 2004). Kuwait is situated in the Middle East on the Arabian Peninsula, bordering the north-western corner of the Arabian Gulf. With an area of 17 818 km², it is slightly smaller than twice the size of Cyprus and approximately twice the size of Puerto Rico. It has a population of 4.5 million inhabitants, one-third of whom are Kuwaiti nationals. Expatriates and foreign workers, mainly from Arabic and South Asian countries, account for the largest proportion of inhabitants (World Population Review, 2020).

*** The problem of the study**

The aim of this study is to estimate the prevalence of both *E. histolytica* and *G. lamblia* according to geographical distribution in the State of Kuwait, and to assess whether factors such as age, sex, nationality and seasonal variation affect the overall number of cases.

*** Advantages of the study**

There are few studies that have investigated parasitic infections in the State of Kuwait, which made working on this manuscript very interesting for us as researchers, as well as challenging and requiring sincere dedication to gather representative data to conduct the analysis.

The study therefore serves as a foundation for further studies and investigation, and we aim to publish our manuscript through a leading open-access online journal to ensure that knowledge-sharing is maximised and to encourage further research in this field.

* Materials and Methods

* Study design

Kuwait is divided into five main public health districts (Al-Amiri, Mubarak, Al-Farwaniya, Al-Jahra and Al-Adan) and one specialised health district (Al-Sabah), which provide health care to all residents in Kuwait (Kuwait Government, n.d.). The population of Kuwait is distributed across the six health districts as follows: 591 856 in Al-Amiri health district, 964 644 in Mubarak health district, 1 213 494 in Al-Farwaniya health district, 572 847 in Al-Jahra health district, 1 028 301 in Al-Adan health district and 274 409 in Al-Sabah specialised health district (Public Authority for Civil Information, n.d.).

Kuwait is a non-endemic country with regard to parasites, but many expatriates come from endemic areas. These expatriates live in some provinces and not others. The present study is a retrospective study of all the samples obtained from all patients presenting with abdominal pain or stomach complaints, and referred for stool parasitological examination to hospitals in the five general health districts (Al-Amiri, Mubarak, Al-Farwaniya, Al-Jahra and Al-Adan) and one specialised health district (Al-Sabah) from January 2018 to July 2019. Hospital records were used to obtain sample findings only, with all patients' personal data anonymised. Patients were referred from both inpatient and outpatient clinics for laboratory stool examination and were diagnosed and treated in these hospitals. The results were recorded on a standardised data collection sheet.

* Sample collection and analysis

All laboratories that work under the Kuwaiti Ministry of Health are well-established units that operate according to the internationally accepted Good Laboratory Practice (GLP). Each patient was given a clean plastic container labelled with the patient's information and asked to provide a fresh stool sample. The containers were then sent to the laboratory with the patients' examination requests. Stool examination indicated the parasitic stage (cyst or trophozoite) if protozoa were present. When multiple samples arrived, samples containing mucous or blood were processed first, followed by watery samples. Stool consistency was used as a guide for whether the trophozoite or cyst stage of the parasite was likely to be present. Table 1 shows the stool consistency and applied techniques.

Table 1: Stool consistency and applied techniques

[1] ample consistency	[2] M ost likely parasitic stage	[3] Technique used		
		[4] aline	[5] odine	[6] T richrome staining
[7] irm	[8] C yst	[9]	[10]	[11] √
[12] oft	[13] C yst/trophozoite	[14]	[15]	[16] √
[17] oose	[18] Tr ophozoite	[19]	[20]	[21] √
[22] atery	[23] Tr ophozoite	[24]	[25]	[26] √

Laboratory tests and examinations were carried out following the procedures and protocols used in laboratory management from the Kuwaiti Ministry of Health in accordance with the WHO guidelines (WHO, 1997). Direct saline smears were used to detect protozoal movement, and Lugol's iodine staining was used to detect parasite structure. A drop of saline was placed on the right half of the slide, while a drop of iodine was placed on the left half of the slide. Stool samples were mixed with the saline and iodine using separate applicator sticks, and then the slide was covered with a coverslip. Specimens were examined systematically, using both low-power (10×) and high-power (40×) magnification, for the presence of pus cells, cysts or trophozoites.

The formalin–ethyl acetate concentration technique was used to determine the presence of parasites. First, 10 mL of formalin was mixed with 1 g of stool by using an applicator stick. The specimen was then filtered into a centrifuge tube until the 7 mL mark was reached. Next, 3 mL of ethyl acetate was added, and the specimen was mixed well for 1 minute. The mixture was centrifuged for 1 minute until a sediment formed. After the fluid had been drained down to the level of the sediment, the specimen was again mixed well. One drop was then transferred to a slide, which was covered with a coverslip and examined microscopically using low-power (10×) and high-power (40×) magnification.

Trichrome staining was also used to determine the presence of parasites. A small amount of stool was transferred using an applicator stick to a slide to form a thin smear. The specimen was fixed using Schaudinn's fixative for 1 hour at room temperature. After the excess fluid had been drained, the slide was placed in 70% ethanol for 1 minute, removed and drained, and then again placed in 70% ethanol for 1 minute. After the excess fluid had been drained, the slide was dipped into a trichrome stain solution for 8 minutes. The slide was then removed, drained and de-stained by dipping it twice into an acetic acid–alcohol solution for 5 seconds in total. The slide was dipped in 95% ethanol for 1–2 seconds, drained, and then dipped again in 95% ethanol for 2–3 seconds. After draining, the slide was dipped in absolute ethanol for 1 minute, and then in xylene for 2–3 minutes. After the slide had been drained, 3–4 drops of mounting medium were added, and the smear was covered with a coverslip and examined microscopically using low-power (10×) and high-power (40×) magnification (WHO, 1997).

*** Statistical analysis**

Epidemiological variables and the prevalences of *E. histolytica* and *G. lamblia* were analysed using SPSS Statistics 25 and the chi-square test, which was used to detect statistical significance regarding geographical distribution and whether factors such as age, sex, nationality and season influenced the number of cases. The probability of a type I error (i.e., alpha level) was set to <0.05. Overall transmission rates of *E. histolytica* and *G. lamblia* in the State of Kuwait were estimated by calculating their transmission rates within each public health district. Comparisons were made between public health districts to determine the district with the highest prevalence of amoebiasis and giardiasis and the groups most vulnerable to these diseases. A chi-square goodness-of-fit test was used to investigate the relationship between parasitic infection and sex, age and nationality under the null hypothesis that the proportion of patients infected with either *E. histolytica* or *G. lamblia* would be equal for each category of sex, age and nationality.

*** Results**

The 6423 stool samples tested from January 2018 to July 2019 were distributed among the hospitals as follows: Al-Amiri Hospital, 1413 samples; Mubarak Hospital, 816 samples; Al-Farwaniya Hospital, 1162 samples; Al-Jahra Hospital, 1221 samples; Al-Adan Hospital, 1554 samples; and Al-Sabah Hospital, 257 samples. The majority ($n = 4817$) (75%) were soft; 642 (10.0%) contained blood; 611 (9.5%) were loose, and 353 (5.5%) were watery. Of the 115 (1.79%) samples that tested positive for intestinal parasites, 69 (60%) were positive for *G. lamblia* and 38 (33%) were positive for *E. histolytica*; *Iodamoeba buetschlii* was found in four stool samples

(3.47%), *Hymenolepis nana* in two samples (1.7%) and *Entamoeba coli* in two samples (1.7%). *G. lamblia* and *E. histolytica* were mostly seen in soft and loose stool samples ($n = 107$). Mixed parasitic infections were seen in six of the infected samples. Two of these samples were positive for both *G. lamblia* and *I. buetschlii*, two for both *G. lamblia* and *E. coli*, one for both *E. histolytica* and *H. nana*, and one for both *E. histolytica* and *I. buetschlii.* Table 2 shows the results of stool microscopy and presence of parasites.

Table 2: Stool microscopy and the presence of parasites

Stool	Parasites present					Total
	None	*G. lamblia*	*E. histolytica*	Other	Mixed	
Soft	4715	63	33	2	4 (2) (2)	4817
Bloody	462	–	–	–	–	462
Watery	349	1	2	–	1	353
Loose	602	5	3	–	1	611

Table 3 shows the distribution of patients with positive results by sex, age and nationality. Males were more likely to be infected than females ($p < 0.001$), and patients older than 15 years were more likely to be infected than those younger than 15 years ($p < 0.001$). With respect to nationality, non-Kuwaiti patients were more likely to be infected than Kuwaiti patients.

Table 3: Chi-square test results for patients positive for E. histolytica or G. lamblia (n = 107) according to sex, age and nationality

[27]	Category	[28] bserved no.	[29] xpected no.	[30] 2	[31] f	[32] -value
[33] ex	[34] ale	[35] 3	[36] 3.5	[37] 4.22	[38]	[39] .000
	[40] emale	[41] 4	[42] 3.5			
[43] ge (years)	[44] –15	[45] 9	[46] 3.5	[47] 4.50	[48]	[49] .000
	[50] 15	[51] 8	[52] 3.5			
[53] ationality	[54] uwaiti	[55] 0	[56] 3.5	[57] .81	[58]	[59] .009
	[60] on-Kuwaiti	[61] 7	[62] 3.5			

Table 4 shows the prevalence of *E. histolytica* and *G. lamblia* and parasite stages in the samples, stratified by hospital. Because of the sample size, the researchers performed chi-square tests using Fisher's exact test. The analysis revealed no significant association between any hospital and parasite stage ($\chi^2 = 2.390$, $p = 0.949$).

Table 4: Prevalence of E. histolytica and G. lamblia in six hospitals

[63] ealth district	[64] G. lamblia		[65] E. histolytica		[66] otal
	[67] yst	[68] rophozoite	[69] yst	[70] rophozoite	
[71] ubarak	[72]	[73]	[74] 7	[75]	[76] 2
[77] l-Adan	[78] 3	[79]	[80]	[81]	[82] 7
[83] l-Farwaniya	[84]	[85]	[86]	[87]	[88] 5
[89] l-Amiri	[90]	[91]	[92]	[93]	[94]
[95] l-Jahra	[96]	[97]	[98]	[99]	[100]
[101] l-Sabah	[102]	[103]	[104]	[105]	[106]
[107] otal	[108] 9	[109] 0	[110] 9	[111]	[112] 07

However, as shown in Table 4, there was a significant association between hospital and type of parasite (i.e., *G. lamblia* or *E. histolytica*) ($\chi^2 = 56.66$, $p < 0.001$). Positive patients who attended Al-Adan Hospital had the highest *G. lamblia* infection rates ($n = 49$, 86.0%), followed by those who attended Al-Farwaniya Hospital ($n = 11$, 73.3%), while positive patients who attended Mubarak Hospital had the highest *E. histolytica* infection rates ($n = 22$, 100%).

The prevalence of *E. histolytica* and *G. lamblia* varied with season; 66 infections occurred in spring and summer (March–August) (57%), and 41 occurred in autumn and winter (September–February) (43%). The most common months for *E. histolytica* and *G. lamblia* infections were April 2019 with 15 infections (14%), May 2018 with 13 infections (12.1%) and August 2018 with 12 infections (11.2%) (see Table 5).

Table 5: Seasonal distribution of E. histolytica and G. lamblia in stool specimens in six hospitals in Kuwait

[113] ear	[114] onth	[115] o. specimens	[116] o. (%) positive	[117] . lamblia	[118] . histolytica
	[120] anuary	[121] 41	[122] (1.76)	[123]	[124]
[119] 018	[125] ebruary	[126] 32	[127] (1.8)	[128]	[129]
	[130] arch	[131] 56	[132] (1.9)	[133]	[134]
	[135] pril	[136] 25	[137] (.6)	[138]	[139]
	[140] ay	[141] 53	[142] 3 (3.7)	[143]	[144]
	[145] une	[146] 25	[147] (0.92)	[148]	[149]
	[150] uly	[151] 40	[152] (1.1)	[153]	[154]
	[155] ugust	[156] 28	[157] 2 (3.35)	[158]	[159]
	[160] eptember	[161] 50	[162] (1.14)	[163]	[164]
	[165] ctober	[166] 45	[167] (0.86)	[168]	[169]
	[170] ovember	[171] 04	[172] (2.64)	[173]	[174]
	[175] ecember	[176] 47	[177] (1.44)	[178]	[179]
	[181] anuary	[182] 28	[183] (1.54)	[184]	[185]
	[186] ebruary	[187] 04	[188] (1.32)	[189]	[190]
	[191] arch	[192] 08	[193] (0.65)	[194]	[195]
[180] 019	[196] pril	[197] 33	[198] 5 (4.5)	[199]	[200]
	[201] ay	[202] 79	[203] (1.07)	[204]	[205]
	[206] une	[207] 16	[208] (0.3)	[209]	[210]
	[211] uly	[212] 23	[213] (1.2)	[214]	[215]
[216] otal	[217]	[218] 243	[219] 07	[220] 9	[221] 8

* Discussion

E. histolytica and *G. lamblia* are typically more prevalent in people of lower socioeconomic status because of a lack of personal hygiene and poor sanitation (Al-Mohammed et al., 2010; Al-Nakkas et al., 2004; Curval et al., 2017). A study conducted by Sarkari, Hosseini, Motazedian, Fararouie and Moshfe (2016) in rural areas of the Boyer-Ahmad district in south-western Iran found that the prevalence of *G. lamblia* was 17.4%. A 2016 study in Libya reported a prevalence of 19.9% for *E. histolytica* and 4.6% for *G. lamblia* infections (Ghenghesh, Ghanghish, BenDarif, Shembesh, & Franka, 2016). Additionally, the European Centre for Disease Prevention and Control (2018) reported that the prevalence of *G. lamblia* infection had increased by 5.3% from 2015 to 2016. In contrast, the overall prevalence of the two intestinal protozoa in this study was only 0.41%, indicating that the State of Kuwait is a non-endemic country because of its high standard of living compared with rural areas mentioned in past studies.

In a 2016 study conducted in northern Jordan, Jaran (2016) found that intestinal protozoan infections were mainly caused by *G. lamblia* (41%) and *E. histolytica* (31%). Similarly, a 2014 study in the Gaza Strip in Palestine showed that the most common cause of intestinal protozoan infection was *E. histolytica* at 28.5%, followed by *G. lamblia* at 9.5% (Mezied et al., 2014). Moreover, a 2011 study in Albania revealed that *G. lamblia* was the most common parasite detected among children (Sejdini et al., 2011). In this study, our results also show that the majority of patients were infected by *G. lamblia* (60%), followed by *E. histolytica* (33%). In contrast, a 2011 study carried out in Makkah, Saudi Arabia, showed that the most common intestinal protozoan was *E. histolytica* (75.8%), followed by *G. lamblia* (21.8%) (Zaglool et al., 2011). A 2015 study in public hospitals in Hail, north-western Saudi Arabia, showed that infections were caused by *E. histolytica* (16.15%) and *G. lamblia* (11.54%) (Amer, Ashakkyty, & Haouas, 2015).

In the current study, the highest numbers of positive cases were concentrated in three health districts: 57 cases (53.2%) at Al-Adan Hospital, 22 cases (20.5%) at Mubarak Hospital and 15 cases (14%) at Al-Farwaniya Hospital. This may be because many expatriates and families with low incomes and levels of education live in these three districts. This is consistent with a 2004 study in Kuwait (Al-Nakkas et al., 2004) and a 2016 study in an urban area in Turkey (Arikan, Gülcan, & Dibeklioğu, 2016).

Regarding the relationship between the intestinal protozoa *E. histolytica* and *G. lamblia* and season, our results show a higher number of infections in spring and summer (March–August) than in autumn and winter (September–February), which is consistent with a study conducted in Riyadh, Saudi Arabia, in 2017 (Amer, Waly, & Al-Zahrani, 2017). Similarly, a 2012 study in the Qassim region of Saudi

Arabia of the monthly distribution of intestinal parasitic infections revealed that infections were highest in June and August (summer) and lowest in December and January (winter) (Imam, Altayyar, Eltayeb, & Almushawa, 2012). Statistically, there was no significant difference between the seasons. The variations in the prevalence of infections between spring/summer and autumn/winter were attributed to the number of patients examined and human exposure to environmental conditions such as temperature and humidity.

*** Conclusion**

The low prevalence of *E. histolytica* and *G. lamblia* parasitic infection in Kuwait is an indicator of the high level of health services provided to the general population. However, there remains room for further development and improvement of strategies aimed at protecting public health. Health authorities in Kuwait could further reduce the number of intestinal parasitic infections through educational and public awareness campaigns.

Finally, health authorities should focus on the number of stool samples tested and diagnostic methods used because some parasitic infections are ruled out following analysis of a single stool sample. This is not sufficient to rule out infection because chronic parasitic infections can be difficult to diagnose. In addition, further detailed surveys and studies are needed to determine the prevalence of intestinal protozoa for different age groups, educational backgrounds, income levels and countries of origin. Asymptomatic patients should also be tested to reveal more accurate prevalence rates of these parasites. Broader research specifically designed to analyse asymptomatic carriers should be implemented using randomised testing.

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