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Seasonal and Circadian Fluctuations of Human Intestinal Parasites in El-Dakahlia Governorate, Egypt

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ABSTRACT

The present study aims to monitor the incidence of human intestinal parasites and their seasonal fluctuations in El-Dakahlia governorate. Human fecal specimens from 428 patients visiting Meet-Ghamr general hospital were monthly collected during different sampling circadian time: morning, afternoon and evening, throughout the period from 2007 to 2009. Microscopic examination was performed using both direct smear and formalin-ether sedimentation techniques. Anti-*Schistosoma* specific antibodies in sera of 279 patients were examined using indirect haemagglutination test. The results demonstrated that the overall prevalence of eight identified intestinal parasites reached, in a descending order,*Entamoebahistolytica* (52.34%), *Schistosomamansoni* (37.63%), *Ascarislumbricoides* (26.86%), *Hymenolepis nana* (16.35%), *Giardia lamblia* (5.14%), *Enterobiusvermicularis* (3.73%), *Trichuristrichiura* (0.46%), and *Trichomonashominis* (0.23%). Highest prevalence was recorded for *E. histolytica*(87.80%) and *G. lamblia* (14.63%) during summer, whereas for *S. mansoni*(73.08%) and*A. lumbricoides* (36.62%) during autumn, and for *H.nana* (22.07%) during winter. Highest incidence was observed in the morning samples for *S.mansoni*(38.27%). Meanwhile, evening samples demonstrated highest incidence for *E. histolytica* (75.41%) and *A. lumbricoides*(36.07%). A marked sex-related difference regarding the infection prevalence was found. Moreover, most of the parasitic infections were the highest in age <=10 years.In conclusion, sampling at a definite circadian time and in a proper month will precisely indicate the parasite prevalence rate. This will consequently optimize monitoring and controlling of the parasite community.

Key words: Seasonal, circadian, intestinal parasites, prevalence, El-Dakahlia governorate.

INTRODUCTION

Parasitic diseases caused by helminths and protozoa are the major causes of human disease and misery in most countries of the tropics and subtropics. They plague billions of people and kill millions annually, and inflict debilitating injuries such as blindness and disfiguration of additional millions (W.H.O., 2012). Parasitic diseases constitute one of the major public health problems for people living in developing countries, especially children who are most severely affected because parasites directly contribute towards malnutrition (Chaudhry*et al*., 2004). High prevalence of infections with intestinal parasites in developing countries is related to poverty, poor living conditions, poor personal and environmental hygiene, inadequate health services, inadequate sanitation and water supply facilities (Cook, 1996; Montresor*et al*., 1998 and Chaudhry*et al*., 2004).

In Egypt, the parasitic diseases are major causes of illness and death in infants, children and adults. For example, the enteric diseases caused by protozoan and helminth parasites rank the second in prevalence to schistosomiasis (EL-Khoby*et al*., 2000). Unfortunately accurate information about the epidemiological picture of parasitic diseases in Egypt is scarce and contradictory.The aim of this study was a trial to demo-nstrate that different chronobiological factors must be considered in the evaluation of human parasites' prevalence. These factors are sampling time throughout the seasons as well as the circadian time of the host himself.

MATERIALS AND METHODS

Study site and sampling strategy:

Human fecal samples (n=428) were monthly obtained in the morning, afternoon and evening from patients visiting urine and stool section in laboratory of Meet-Ghamer general hospital, Dakahlia governorate along the period 2007 to 2009. The specimen bottles collected from the patients were labelled with patient name, occupation, age and sex. Patients were divided into 4 age-categories: 1-10, 10-20, 20-30 and over 30 years, as shown in table (1).

Table (1): Sample size according to different considered factors.

Factor	Host sex		TOTAL	Age category				TOTAL
Season	Male	Female		<=10 years	10-20 years	20-30 years	>30 years	
Winter	63	82	145	47	19	29	50	145
Spring	41	89	130	36	31	19	44	130
Summer	27	55	82	30	13	12	27	82
Autumn	38	33	71	19	11	19	22	71
TOTAL	169	259	428	132	74	79	143	428

1. **Stool collection and parasitological examination:**

Microscopical examination was done by two methods for confirmation of the presence of infection; direct smear and formalin-ether sedimentation according to Soulsby (1982).

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2. Antibody test for schistosomiasis:

Sera were monthly collected in the morning, afternoon and evening from 279 patients and examined for antibody detection by indirect haemagglutination test following the procedures of the manufacturer.

3. Data analysis:

Epidemiological characteristic represented in the infection prevalence was calculated according to Bush *et al*. (1997) for analyzing the factors considered in this study.

RESULTS

Results revealed the presence of eight intestinal parasitic species in the study area. Their prevalence in a descending order were as following: *E. histolytica* (52.34%) and *S. mansoni* (37.63%), *A. lumbricoides* (26.86%), *H. nana* (16.35%), *G. lamblia* (5.14%), *E. vermicularis* (3.73%), *T. trichiura* (0.46%), and *T. hominis* (0.23%), as shown in figure (1).

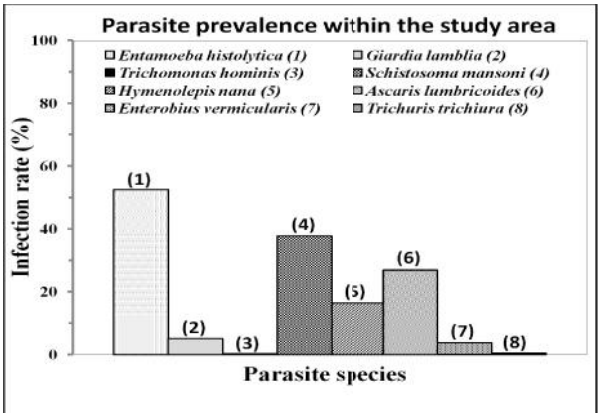

Figure (1): Prevalence of the intestinal parasites recorded in the study area.

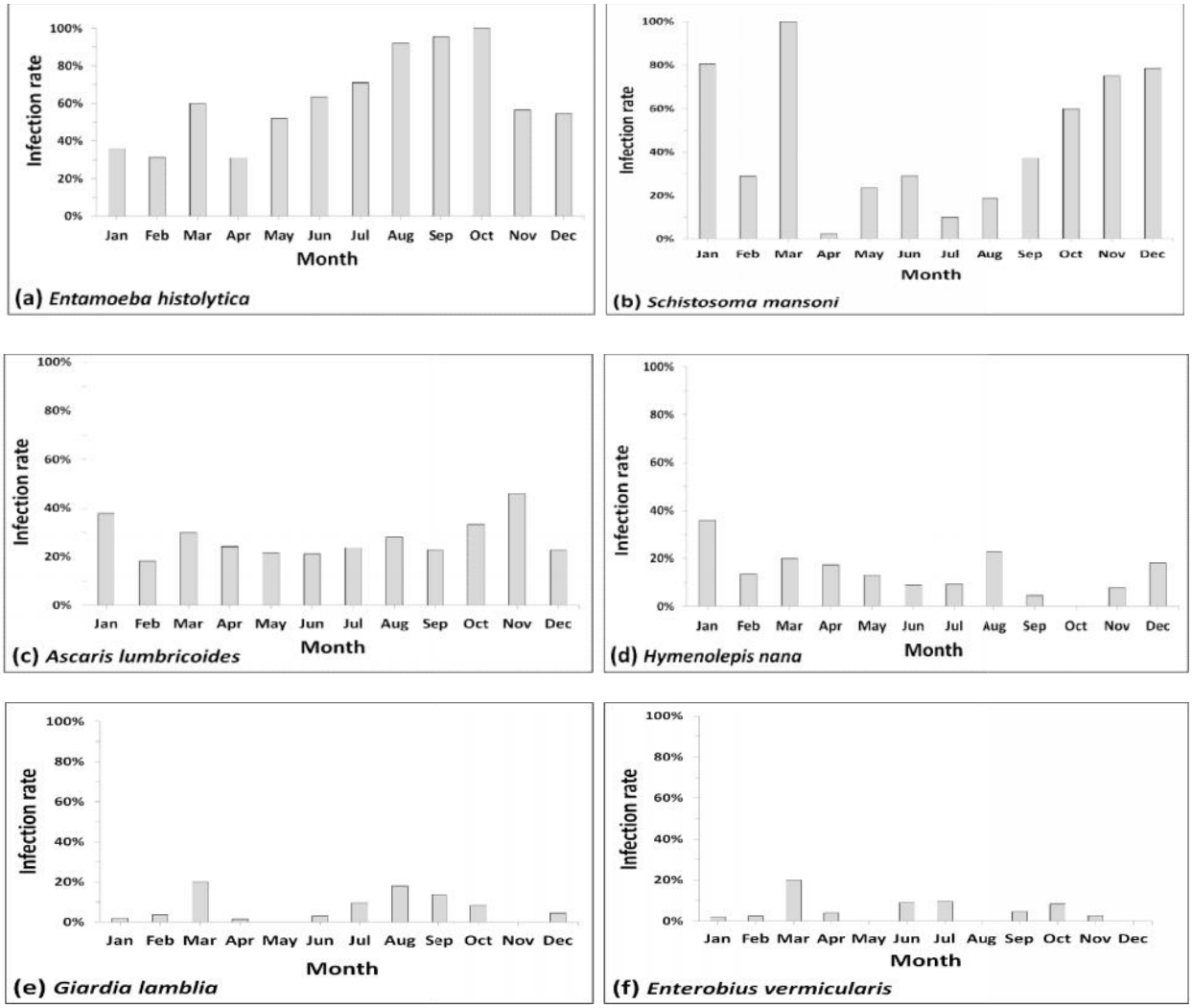

Figure (2): Monthly prevalence of the parasites throughout the year in Meet-Ghamr city, El-Dakahlia governorate.
Note: Both *Trichomonashominis*and *Trichuristrichiura*are not included because they were rare in the study area.

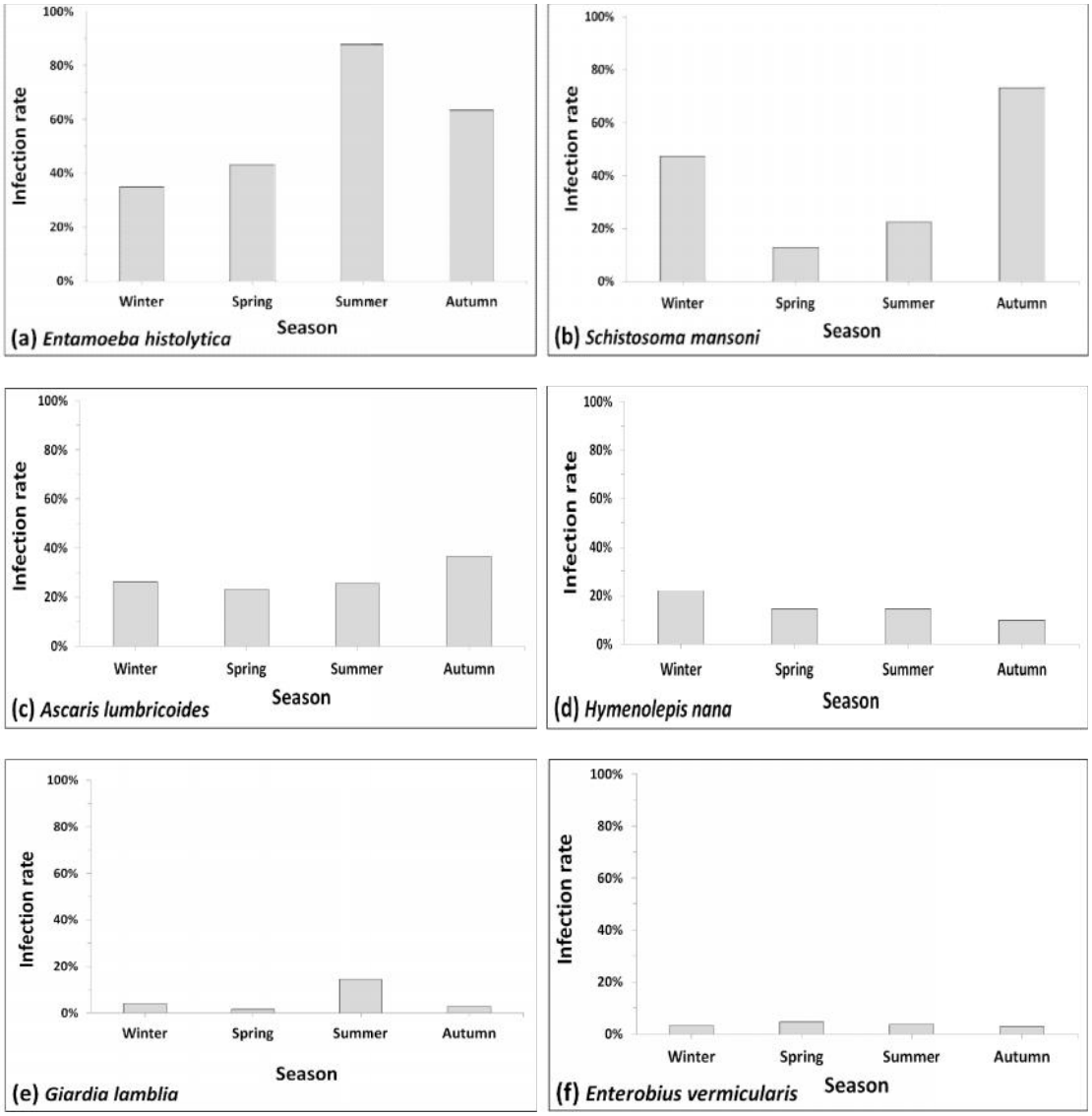

Figure (3): Seasonal prevalence of the parasites throughout the seasons in Meet-Ghamr city, El-Dakahlia governorate. Note: Both *Trichomonashominis*and *Trichuristrichiura*are not included because they were rare in the study area.

1. **Monthly and seasonal variation of the infection:**

As shown in figures (2 and 3), the monthly and seasonal fluctuations illustrated that some parasites recorded its highest prevalence in summer such as *E. histolytica* (87.80%) and *G. lamblia* (14.63%) (Fig. 3a and e), whereas others demonstrated its highest prevalence in winter such as *H. nana* (22.07%)(Fig. 3d). Meanwhile, data elucidated that *S. mansoni* and *A. lumbricoides* have the highest prevalence in autumn (73.08% and 36.62%, respectively) (Fig. 3b and c).

2. **Circadian time variation of the infection:**

Circadian time of the host appeared to play a role in the infection prevalence of the intestinal parasites under investigation. Evening sampling was characterized by highest prevalence of *E. histolytica* (75.41%) and *A. lumbricoides* (36.07%) (Tables 2a and c). On the other hand, afternoon sampling revealed highest prevalence of *H. nana* (33.33%), *G. lamblia* (22.22%) and *E. vermiculoris*(11.11%), as shown in tables (2 d, e and f). Morning sampling recorded high prevalence of *S. mansoni* (38.27%) (Table 2b).

3. **Host occupation, age and sex variations of the infection:**

Table (3) shows the variation in the prevalence of the parasitic infections according to the host occupation, age category andsex. Results showed that the highest infection prevalence was recorded in males as compared to females, except for *H. nana* where the prevalence was the highest in females. The prevalence of *E. histolytica*, *H. nana,G. lamblia* and *E. vermicularis* was the highest among patients <= 10 years. The infection prevalence of *S. mansoni* and *A. lumbricoides* reached the highest among patients from 20-30 years and 10-20 years, respectively.The highest prevalence was recorded among farmers, except for *G. lamblia* where the infection was only recorded among the student.

Table (2): Prevalence of the intestinal parasites versus sampling time. Note: Both *Trichomonashominis*an *Trichuristrichiura*are not included because they were rare in the study area.

(a) *Entamoeba histolytica*

Sampling Time	Examined	Infected	Non Infected	Percentage
Morning	349	165	184	47.28%
Afternoon	18	13	5	72.22%
Evening	61	46	15	75.41%
Total	428	224	204	52.34%

(b) *Schistosoma mansoni*

Sampling Time	Examined	Infected	Non Infected	Percentage
Morning	243	93	150	38.27%
Afternoon	6	2	4	33.33%
Evening	30	10	20	33.33%
Total	279	105	174	37.63%

(c) *Ascaris lumbricoides*

Sampling Time	Examined	Infected	Non Infected	Percentage
Morning	349	89	260	25.50%
Afternoon	18	4	14	22.22%
Evening	61	22	39	36.07%
Total	428	115	313	26.87%

(d) *Hymenolepis nana*

Sampling Time	Examined	Infected	Non Infected	Percentage
Morning	349	53	296	15.19%
Afternoon	18	6	12	33.33%
Evening	61	11	50	18.03%
Total	428	70	358	16.36%

(e) *Giardia lamblia*

Sampling Time	Examined	Infected	Non Infected	Percentage
Morning	349	15	334	4.30%
Afternoon	18	4	14	22.22%
Evening	61	3	58	4.92%
Total	428	22	406	5.14%

(f) *Enterobius vermicularis*

Sampling Time	Examined	Infected	Non Infected	Percentage
Morning	349	9	340	2.58%
Afternoon	18	2	16	11.11%
Evening	61	5	56	8.20%
Total	428	16	412	3.74%

Table (3): Prevalence of the intestinal parasites versus host occupation, age category and sex.
Note: Both *Trichomonas hominis*and *Trichuristrichiura*are not included because they were rare in the study area.

(a) *Entamoeba histolytica*

Factor	Occupation				Age category (years)				Sex	
	Employee	Housewife	Farmer	Student	>30	20-30	10-20	<=10	Female	Male
No. Examined	64	89	86	187	143	79	74	132	259	169
No. infected	25	40	52	105	71	34	41	78	127	97
Prevalence of infection%	39.06	44.94	60.47	56.15	49.65	43.04	55.41	59.09	49.03	57.40

(b) *Schistosoma mansoni*

Factor	Occupation				Age category (years)				Sex	
	Employee	Housewife	Farmer	Student	>30	20-30	10-20	<=10	Female	Male
No. Examined	57	85	84	53	135	74	53	17	167	112
No. infected	16	25	56	8	54	37	14	0	62	43
Prevalence of infection%	28.07	29.41	66.67	15.09	40.00	50.00	26.42	0	37.13	38.39

(c) *Ascaris lumbricoides*

Factor	Occupation				Age category (years)				Sex	
	Employee	Housewife	Farmer	Student	>30	20-30	10-20	<=10	Female	Male
No. Examined	64	89	86	187	143	79	74	132	259	169
No. infected	9	11	29	66	23	16	33	43	66	49
Prevalence of infection%	14.06	12.36	33.72	35.29	16.08	20.25	44.59	32.58	25.48	28.99

(d) *Hymenolepis nana*

Factor	Occupation				Age category (years)				Sex	
	Employee	Housewife	Farmer	Student	>30	20-30	10-20	<=10	Female	Male
No. Examined	64	89	86	187	143	79	74	132	259	169
No. infected	1	1	4	64	1	4	10	55	47	23
Prevalence of infection%	1.56	1.12	4.65	34.22	0.70	5.06	13.51	41.67	18.15	13.61

(e) ***Giardia lamblia***

Factor	Occupation				Age category (years)				Sex	
	Employee	Housewife	Farmer	Student	>30	20-30	10-20	<=10	Female	Male
No. Examined	64	89	86	187	143	79	74	132	259	169
No. infected	0	0	0	22	0	0	1	21	10	12
Prevalence of infection%	0	0	0	11.76	0	0	1.35	15.91	3.86	7.10

(f) ***Enterobius vermicularis***

Factor	Occupation				Age category (years)				Sex	
	Employee	Housewife	Farmer	Student	>30	20-30	10-20	<=10	Female	Male
No. Examined	64	89	86	187	143	79	74	132	259	169
No. infected	1	1	3	10	2	2	4	8	9	7
Prevalence of infection%	1.56	1.12	3.49	5.35	1.40	2.53	5.41	6.06	3.47	4.14

DISCUSSION

The prevalence rates of the investigated parasites were ranging from high to rare. The most prominent pathogenic parasite was *E. histolytica*(52.34%). Similar high incidence has been reported; it reached in Menufiagovernorate 21.7% (Abd-Alla*et al*., 2000) and in Gharbia governorate 37.5% (Abo-Al-Azm*et al*., 1997).

Microscopic stool examination is not an effective method for detection of *S. mansoni*. Results revealed that the infection was recorded only in one specimenwith prevalence of (0.23%).Contrarily,using antibody detection indicated that its prevalence reached (37.63%). Therefore, the antibody tests for schistosomiasis may be much effective than the classic stool examination. The observed *S. mansoni*high incidence was within the previously reached range. El-Khoby*et al*. (2000) reported that the average prevalence range in 5 governorates in Lower Egypt was (17.5-42%). Diagnosis of schistosomiasis by detection of specific antibodies is likely to be more sensitive than the traditional method of diagnosis by detection of eggs in stool or urine (Hamilton *et al.,* 1998). A possibility of detection of past infection using the immunological technique is taking into consideration.

*A. lumbricoides*was recorded in high prevalence rate (26.86%). It reached (27.31%) in Menoufia governorate (Bakr*et al*., 2009) whereas Abo-Al-Azm*et al*. (1997) recorded 6.2% in Gharbia governorate and El-Shazly*et al*. (2006) recorded only 1.8% in Dakahlia governorate.Different prevalence rates for *H. nana* were previously recorded: (3.9%) in Dakahlia governorate (El-Shazley*et al*., 2006), (2.96%) in Menoufeia governorate (Bakr*et al*., 2009) and (6.2%) in Gharbia governorate (Abo-Al-Azm*et al*., 1997). Meanwhile, the present results revealed a higher rate (16.35%).During the present study, *G. lamblia*was recorded in low rates (5.14%) compared with that recorded in Gharbia governorate (27.8%) (Abo-Al-Azm*et al*., 1997), in Dakahlia governorate (19.6%) (El-Shazly*et al*., 2006) as well as in Menoufia governorate (10%) (Bakr*et al*., 2009).

The incidence of infection of *E. vermicularis*was (3.73%) in the present work. Nevertheless, this parasite was recorded in higher prevalence in Gharbia governorate (9.6%) (Abo-Al-Azm*et al*., 1997) and in Dakahlia governorate (4.1%) and (3.9%) (El-Shazly*et al*., 2006). Routine stool examination methods, such as formalin-ether concentration showed that only a limited portion of *E. vermicularis* infections can be detected as compared to the anal swab test (Lee *et al*., 2000). Consequently, the present data may be just an indicator for a higher prevalence of *E. vermicularis* in the study area.

The presence of rare species in the study area, e.g. *T. hominis*(0.23%)and *T. trichiura* (0.46%), was observed in the current work. Meanwhile low incidence rate of *T. hominis* (4.2%) in El-Mansoura was documented by El-Shazly*et al*. (2006). For *T. trichiura,* low rates were also recorded by El-Shazly*et al*. (2006) in Dakahlia governorate (0.7%) and by Abo-Al-Azm*et al*. (1997) in Gharbia governorate (2.4%).This low rate may be due to the improvement of the socio-economic status. Meanwhile applying colonoscopy examination may be helpful for better diagnosis as being applied by Kim *et al*. (2003) and Ok*et al.* (2009).

Generally, depending on the temperature, humidity and various environmental factors, parasite prevalence rate could be influenced. High and low incidence of intestinal parasites in different Egyptian governorates may be due to socio–demographic and environmental conditions, also unsafe water supply and unhygienic personal habits. High incidence may always be attributed to the rural areas since the socio-economic, hygienic conditions and medical services were relatively less in the rural than urban areas (Mohammad *et al*., 2012).

Omar (2002) and Olusgum*et al*. (2011) reported that seasonal and environmental parameters may have a role in prevalence of parasites. In addition, Sharif (2002)mentioned that the highest incidence of parasites occurred in summer while, the lowest incidence of parasites evident in winter. He attributed the peak incidence of intestinal parasites in summer to the increase in contamination of drinking and swimming waters and irrigation of vegetable by waste water. In this study, highest prevalence recorded during summer (e.g. *E. histolytica*and*G. lamblia*) as well as in winter (e.g. *H. nana*) indicating that season may have a role to assess the parasite incidence. It is suggested that the adaptive importance of the "gate" mechanism is associated with the concentration of *S. mansoni*cercariain the water at times when the vertebrate is present, optimizing the contact between the parasite and the host (Bogéa*et al*., 1996).

Circadian time of the host must be considered when applying different diagnostic methods for the detection of parasites in biological samples ranging from traditional to more recent molecular methods. Sampling time must be taken into account (López*et al*., 2007 and

Manguin*et al*., 2010). Current results confirmed high prevalence of evening sampling (e.g. in *A. lumbricoides*) or afternoon sampling (e.g. in *G. lamblia*), therefore the host circadian time must be considered in diagnosing the parasite incidence.Doehring*et al*. (1983) confirmeda circadian rhythm of *S. haematobium* egg excretion, with a peak around noon.The cercarial emergence pattern of *S. mansoni* from Oman is circadian, exhibiting either a diurnal or a nocturnal phenotype (Mouahid*et al*., 2012).

Results showed a marked sex-related difference regarding the infection prevalence with the intestinal parasites, where the males were slightly susceptible to the parasitic infection as compared to the females; exception for *H. nana*. Al-Naemy*et al*. (2012) attributed the high prevalence of gastrointestinal parasites in males to their usual behaviour by spending more time outdoors in road and streets playing or even working. In contrast, susceptibility to the parasitic infection in male hosts was attributed to testosterone. Testosterone may increase the movement, display rates and aggression of the host, which can lead to higher exposure to parasites (Klein, 2000). In addition, it may increase susceptibility to infection or infestation by directly lowering the immunocompetence of the individual, via suppression of the immune system (Ulter and Olsson, 2003). Barnard *et al*. (2002) correlated reduced resistance and thus greater parasite intensities in rodents to the larger adrenal glands, testes and seminal vesicles of the host. In the present study, the prevalence of the intestinal parasites in males was slightly higher than females indicating the possible effects of the usual behaviour of the individual.

Gastrointestinal protozoa and helminths flourish in areas characterized by warm temperatures, humidity, poor sanitation, dirty water, and substandard and crowded housing (Harhay*et al*., 2010). Unfortunately, this is the case in the study area. Most of the parasitic infections recorded in this study were the highest in age ≤ 10 yearsindicating firstly the poor health education in the surrounding community and secondly the susceptibility of young individuals than older one to the infection. Interestingly, age–related difference was mainly parasitic-species dependent. Rayan*et al*. (2010) found age–related differences in their study about parasitic infestations in school children.

In conclusion, this study is an attempt to focus on the concept of chronobiological approach in sampling periods which may help in evaluating accurate survey of parasite incidence in Egypt. The current preliminary results indicated that investigating the host circadian time and the sampling month may have a crucial role to get proper infection incidence of parasites in Egypt and consequently optimize monitoring and controlling of the parasite community. Therefore, detailed further studies are necessary for analyzing the Egyptian parasitic fauna.

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التغيرات الموسمية واليومية للطفيليات المعوية في الانسان في محافظة الدقهلية – مصر

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الملخـــص العربـــي

تهدف هذه الدراسة الى رصد معدلات تواجد الطفيليات المعوية وتغيراتها الموسمية فى محافظة الدقهلية. ولقد تم تجميع عينات البراز من ٤٢٨ مريض خلال زيارتهم لمستشفى ميت غمر العامة، وذلك فى أوقات مختلفة خلال اليوم : فى الصباح، والظهيرة والمساء، وذلك خلال الفترة من ٢٠٠٧-٢٠٠٩. ولقد تم إجراء الفحص الميكروسكوبى للطفيليات باستخدام تقنية المسحة المباشرة وتقنية الترسيب باستخدام الفورمالين والايثر وكذلك تم تشخيص الأجسام المضادة فى عينات المصل في ٢٧٩ من المرضي باستخدام تقنية "التراص الدموي".

لقد أظهرت النتائج وجود ثمانية أنواع من الطفيليات فى منطقة الدراسة حيث بلغ معدلات انتشارها كالتالى: انتاميبا هستوليتكا (52.34%) ، البلهارسيا المعوية (37.63%) ، الأسكاريس (26.86%) ، هيمينوليبس نانا (16.35%) ، جياردیا لامبليا (٥.١٤%) ، الانتروبيس فرميكيولاريس (3.73%) ، تراكيورس تراكيورا (0.46%) ، وتريكوموناس هومينيس (0.23%). وأوضحت النتائج أن أعلي معدلات إصابة تم تسجيلها خلال موسم الصيف لطفيل الانتاميبا هستوليتكا (٨٧.٨%) وطفيل الجيارديا لامبليا (١٤.٦٣%) بينما بلغت أعلى معدلات الإصابة بالبلهارسيا (٧٣.٠٨%) والإسكاريس (٣٦.٦٢%) خلال موسم الخريف ولطفيل هيمينوليبس نانا (٢٢.٠٧%) خلال موسم الشتاء. ولوحظ أن أعلي معدلات للإصابة فى العينات التى تم تجميعها صباحاً كانت لطفيل البلهارسيا (٣٨.٢٧%) وفى فترة الظهيرة كانت لطفيل الجيارديا لامبليا (٢٢.٢٢%) وهيمينوليبس نانا (٣٣.٣٣%) والانتروبيس فرميكيولاريس (١١.١١%)، بينما بلغ أعلي معدل إصابة فى عينات المساء لطفيل الانتاميبا هستوليتكا (٧٥.٤١%) والاسكاريس (٣٦.٠٧%). وقد وجد اختلافاً ملحوظاً في معدل انتشار الاصابةطبقا لجنس المصاب ، علاوة علي ذلك لوحظ أن أعلي الاصابات قد انحصرت في الشريحة العمرية (<= ١٠سنوات).

خلصت النتائج من الدراسة الحالية الى أن تجميع وتشخيص العينات فى وقت محدد خلال اليوم والشهر سيعطى دلالة أكثر دقة على معدلات انتشار الطفيليات. وهذا بالتالى يرتقى بمستوى دراسات الرصد ومكافحة انتشار مجتمعات الطفيليات. وبناءاً عليه فمن الضروري إجراء مزيد من الدراسات التفصيلية لتحليل بيئة الطفيليات المصرية.