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COMPARISON BETWEEN KATO-KATZ THICK SMEAR AND SEDIMENTATION TECHNIQUES IN DIAGNOSIS OF FAECAL-ORALLY TRANSMITTED HELMINTHES AND OTHER GEOHELMINTHES

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Abstract

Kato-Katz technique is widely used for the diagnosis of Fecal-orally transmitted helminthic infections. It is relatively simple and inexpensive. However, a single slide prepared from a single stool specimen has low sensitivity, particularly in light infections. Therefore, there is a great need for concentration techniques. This study detected an accurate and affordable method for diagnosis of fecal-orally transmitted helminthes and other geohelminthes.

The study was carried out on 217 stool samples of different sex and age groups. Stool samples were collected from different urban and rural areas in Sharkia Governorate. Stool samples were examined macroscopically and analyzed by different parasitological techniques: direct wet mount (DWM), Kato-Katz thick smear, spontaneous sedimentation in tube technique (SSTT) and formol ether sedimentation technique (FEC).

The results showed that formol ether sedimentation method detected 59 positive samples followed by spontaneous sedimentation in tube technique (48/59 positive samples). FEC showed significant difference when compared to direct wet mount and Kato-Katz thick smear. SSTT also showed significant difference when compared to DWM ($P < 0.05$).The overall prevalence of intestinal helminthes was (29.6%) among studied samples in Sharkia Governorate. The commonest helminthic infection was *H. nana* (12%) followed by *E. vemicularis*(10%) then *A. lumbricoides* (3.7%).

Keywords: Geohelminthes, Kato-Katz thick smear, direct wet mount methods, Formol ether concentration method.

Introduction

Helminthic infections, which have direct life cycle, do not need an intermediate host to infect a new host and spread via fecal contamination of food and drinks are often referred to as faecal-orally transmitted helminthes. Infection acquired by direct ingestion of infective eggs is powerfully linked with the level of personal hygiene and sanitation in the community. The lack of latrines and adequate sewage disposal facilities has been known to contribute to the spread of the infective stages of these parasites thus bringing about a widespread contamination of food and drinks. Infections can also be acquired through contaminated unwashed hands, insects, and circulation of bank notes and/or by the wind in dry conditions (Idowu and Rowland, 2006).

Ascaris lumbricoides (roundworm) is one of the most common and most widespread human infections, affecting about 1.2 million people worldwide. (Bethony *et al,* 2006). *Ancylostoma duodenale* frequently overlap *Necato ramericanus* infections in areas of Africa, India and China. Approximately 740 million individuals were currently infected worldwide with *A. duodenale* and *N. americanus* (Sheorey *et al,* 2007).

Trichuris trichiura is most common in warm, moist, tropical and sub-tropical countries. An estimated 604 to 795 million persons harbor *T. trichiura*. Typically the most severe infections are in children aged 5 to 15 years, with a decline in intensity and frequency in adulthood (Bethony *et al,* 2006).
Enterobius vermicularis (Pinworm) has the widest distribution of any parasitic helminth, and it is estimated that approximately 200 million people are infected internationally. It has become the most common intestinal parasite seen in a primary care setting, regard-

less of factors such as race, socioeconomic status and culture. As such, pinworm serves as an exception to the general rule that intestinal parasites are uncommon in high level societies (Burkhart and Burkhart, 2005).

Strongyloides stercoralis is zoonotic intestinal nematode that infects tens of millions of people worldwide. *S. stercoralis* is unique among intestinal nematodes in its ability to complete its life cycle within the host through an asexual autoinfective cycle, thus infection persist in the host indefinitely. In immunocompromised patients, this autoinfective cycle can become amplified into a potentially fatal hyperinfection syndrome, characterized by increased numbers of infective filariform larvae in stool and sputum and clinical manifestations of the increased parasite burden and migration, such as gastrointestinal bleeding and respiratory distress (Keiser and Nutman, 2004).

Hymenolepis nana is the commonest cestode in humans with infection prevalence highest among children encountered worldwide and in warm climates with poor sanitation facilities (Schantz, 2006).

The Kato-Katz technique is widely used for the diagnosis of intestinal helminthes. Its main advantage is that it is relatively simple and inexpensive, even under field conditions. It produces semi-quantitative egg counts that can be used as indicator of infection intensity. However, if only a single Kato-Katz slide was prepared from a single stool specimen, sensitivity was reduced, particularly in light infections. This led to a marked underestimation of the prevalence of infection especially when infection intensity is low (Lier *et al,* 2008).

The present study aimed to detect an accurate and affordable method for diagnosis of faecal-orally transmitted helminthes and other geohelminthes.

Materials and Methods

Type of the study: Cross sectional study.

Study population: This study was carried out on 217 stool samples of different sex and age groups. Stool samples were collected from different urban and rural areas in Sharkia Governorate. The study was carried out during the period from March 2014 to April 2015.

Medical history was taken as regard age, sex, residence (rural or urban), complaint (GIT disturbances as diarrhea, abdominal pain, anorexia nausea, vomiting and perianal itching), relevant risk factors (previous contact with soil, adequacy of hand hygiene, walking bare-footed, untrimmed nails or ingestion of unwashed vegetables or fruits) and past history and family history of similar condition.

Fresh stool samples were collected in a clean dry leak-proof plastic container, uncontaminated with urine, specimens were examined within one hour of being collected, by macroscopic examination with the naked eye for determination of stool consistency, color, odor, blood, and mucus and by microscopic examination for detection of parasitic infection as follows: 1- Direct Smear Method a- Unstained smear (Melvin and Brooke, 1985), and b- Lugol's iodine stained smear (Chessbrough, 2005). 2- Kato Katz thick smears method (Katz *et al,* 1972). 3- Spontaneous sedimentation in tube technique (SSTT) (Tello and Canales, 2000). And 4- Formol-ether sedimentation technique (Ritchie, 1948)

Statistical analysis: Data were collected throughout questionnaires were coded, entered and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) software for analysis. According to the type of data, a specific test was used to test differences for significance. Differences between frequencies (qualitative variables) in groups were compared by Chi-square test.

Ethical considerations: The study was approved by the Ethics Committee of the Faculty of Medicine, Zagazig University, and all individuals or their legal guardian signed an informed consent form.

Results

The results were given in tables (1, 2, 3 & 4) and in figures (1, 2, 3, 4, 5, 6, 7, 8, 9 & 10).

Table 1: Prevalence of intestinal helminthes among samples.

Helminthes	Total examined : 217	
	Positive samples	Percentage
Hymenolepis nana	26	12
Enterobius vermicularis	22	10
Ascaris lumbricoides	8	3.7
Ancylostoma duodenale	2	0.9
Heterophyes heterophyes	2	0.9
Strongyloides stercoralis	1	0.46
Schistosoma mansoni	1	0.46
Trichocphalus trichiura	0	0
Total infected	62	28.6

Table 2: Prevalence of faecal-orally transmitted helminthes & other geohelminthes among samples by different parasitological methods & agreement between them.

Method	DWM		K.K.		SSTT		FEC		X^2	P
Helminthes	N	%	N	%	N	%	N	%		Value
A. lumbricoides	2	0.9	4	1.8	6	2.7	8	3.7	2.22	0.52
A. duodenale	0	0.0	0	0.0	1	0.5	2	0.9	1.62	0.65
T. trichiura	0	0.0	0	0.0	0	0.0	0	0.0	0.0	1.0
E. vermicularis	5	2.3	10	4.6	18	8.0	22	10.0	5.6	0.13
S. stercoralis	0	0.0	0	0.0	1	0.46	1	0.46	0.92	0.82
H. nana	12	5.5	11	5.07	22	10.0	26	12.0	3.9	0.26
Total	19	8.7	25	11.05	48	22.1	59	27.0	13.3	0.003

Regard total: DWM & KK, P=0.59, DWM & SSTT P=0.01*, DWM & FEC, P=0.002*, KK & SSTT, P=0.054 KK & FEC, P=0.009*, SSTT & FEC P = 0.48 (* means significance)

Table 3: Performance and validity of DWM, K.K. thick smear and SSTT in relation to FEC as a gold standard.

Method	Results	FEC				Total	X^2	P Value	Sensitivity
		+ve		-ve					
		No.	%	No.	%				
DWM	+ve	19	32.2	0	0.0	19	55.7	0.0**	32.2%
	-ve	40	67.8	158	100	198			
KK	+ve	25	45.7	0	0.0	25	75.6	0.0**	45.7%
	-ve	34	54.3	158	100	192			
SSTT	+ve	48	81.3	0	0.0	48	165	0.00**	81.3%
	-ve	11	18.7	158	100	169			
Total		59	100	158	100	217			

Table 4: Relationship between number of collected stool samples per individual and each parasitological method.

Stool sample/person		Total +ve	DWM		K.K.		SSTT		FEC	
No.	frequency		+ve	frequency	+ve	frequency	+ve	frequency	+ve	frequency
	72	3	3	3	3	1	3	12	3	22
			2	6	2	8	2	18	2	14
			1	5	1	11	1	3	1	0
2	36	17	2	4	2	3	2	4	2	9
			1	1	1	2	1	9	1	8
1	109	6*	1	0	1	0	1	2	1	6
Total	217	59	19		25		48		59	
X^2		37.24								
P Value		0.00**								

Discussion

Intestinal parasitic infections still constitute one of the major causes of public health problems in the world, particularly in developing countries. Records showed increasing trends in helminthiasis, particularly in the developing nations (Mohammad *et al,* 2012). Inadequate water supply, absence of correct sanitation and overcrowded living conditions combined with the lack of access

to correct health care and low level of education make the poor particularly susceptible to infection and disease, including helminthiasis. It is estimated that approximately one third of three billion people that live below poverty line in developing regions of the Sub-Saharan Africa, Asia and the Americas are infected with one or more helminthes (Lone *et al,* 2011).

In the present study, the overall prevalence of intestinal helminthes was (28.6%). This rate was nearly similar to that of Hamed *et al*. (2013), and Hegazy *et al.* (2014) who recorded (31.8%), and (22.6%) in Sohag Governorate and Damanhur City respectively. On the other hand, higher prevalence was recorded in other previous studies as reported by El-Hamshari (1994), Younis *et al.* (1996) who reported a rate of (54.4%), (40.4%) in Qualyobia Governorate, the outpatient clinic of the Fakous General Hospital respectively.

In the current study, the prevalence rate of *A. lumbricoides* was (3.7%).This rate agreed with Mohammad *et al*. (2012) and Zaytoun *et al.* (2013) who reported a rate of (5.2%) and (5.8%) among school children in Damietta and in Qena Governorates . However, lower prevalence of *Ascaris* was reported by Hamed *et al.* (2013) who reported a rate of (0.2%) among rural population in Sohag Governorate. Lower prevalence in this study was attributed to dry sandy soil which is unsuitable for *Ascaris* egg development, dry climate and wide use of insecticides and chemical fertilizers. On the other hand, higher prevalence of *Ascaris* in Alexandria Governorate was reported by Abo-Elfotouh *et al.* (1995) who recorded a rate of (24.4%) and Fawzi *et al.* (2004) who reported that *A. lumbricoides* was the most prevalent vegetable-transmitted nematode parasite with an infection rate of (18.4%).

In the present study, the prevalence rate of *A. duodenale* was (0.9%). This low prevalence may be explained by the fact that *Ancylostoma* larva requires appropriate humidity and warmth to survive and hence transmission. Also, good personal hygiene and habits as wearing shoes may be additional factors. Safar and Eldash (2015) in Fayoum Governorate reported a total of (26.1%) for *A. lumbricoides* and *A. doudenale* together in Fayoum G. schoolchildren, which might be due to the fact that the climatic conditions favor these nematodes.

In the present study, the prevalence rate of *E. vermicularis* was (10%). This prevalence might be underestimated as the standard diagnostic approach, scotch tape method, wasn't done and performed parasitological methods detect only 10-15% of *Enterobius* infected cases (Mac-Clements, 2015). The present prevalence rate of *E. vermicularis* agreed with Zaytoun *et al.* (2013) who reported a rate of (8%) in Qena Governorate. On the other hand, much higher prevalence of *E. vermicularis* was in Sohag Governorate, Hamed *et al.* (2013) reported a rate of (21.7%) among rural population. However, some studies showed significantly lower prevalence as in Damietta Governorate, Mohammad *et al*. (2012) recorded a rate of (5.2%) among school children.

In the present study, the prevalence of *S. stercoralis* is (0.46%). It may be underestimated due to the fact that strongyloidiasis is one of the most difficult parasitic diseases to diagnose due to general absence of distinctive ova in stool samples, low parasite load, rarity of diagnostic rhabditiform larvae in stool, asymptomatic or non-specific presentation, particularly in uncomplicated cases and the need for special diagnostic techniques like stool culture (Bisoffi *et al,* 2013).

Prevalence rate of *H. nana* in the current study (12%) agreed with Ibrahium (2011) recorded a rate of (12.5%) among rural primary school pupils in El-Minia Governorate. Also, Hamed *et al.* (2013) reported a rate of (9.9%) among rural population in Sohag Governorate. On the other hand, lower prevalence was obtained by Mohammed *et al*. (2012) who recorded a rate of (3.2%) among school children in Damietta Governorate. Also, Safar and Eldash (2015) reported *H.*

nana in males (16.1%) & females (11.5%) in school children in Fayoum Governorate. Moreover, Cabada *et al*. (2016) in in Peru stated that hymenolepiasis is a common gastrointestinal helminth in the Cusco region associated with significant morbidity in children in rural communities. They added that the impact caused by the emergence of *H. nana* as a prevalent intestinal parasite deserves closer scrutiny.

In the current study, the highest prevalence of fecal-orally transmitted helminthes was detected by the formol ether concentration method with the highest sensitivity, followed by spontaneous sedimentation in tube technique then Kato-Katz thick smear with the rates of 27%, 22.1% and11.05% respectively. On the other hand, the direct wet mount was the least sensitive (8.7%). FEC showed significant difference when compared to direct wet mount and Kato-Katz thick smear. This was in concordance with Uga *et al.* (2010), Tay *et al.* (2011) and Funk *et al*. (2013) and Eltayeb *et al*. (2016). However, Goodman *et al.* (2007), Glinz *et al.* (2010), Swamy *et al.* (2013), Endris *et al.* (2013), Funk *et al*. (2013) and Nikolay *et al*. (2014) reported higher prevalence rate of soil-transmitted helminthes by Kato-Katz thick smear than that by formol ether concentration method.

In the present study, when two or three stool samples were collected per individual, more positive cases were detected with very highly significant difference as compared to those detected after collection of only one sample. This was in agreement with Steinmann *et al.* (2008) who reported that analysis of 3 instead of a single stool specimen considerably increased the sensitivity of the diagnostic tools. Also, Knopp *et al*. (2008) stated that increased sampling effort result in higher observed helminthes prevalence. Forsell et al. (2016) in Zanzibar (Tanzania) stated that the carriage of intestinal parasites was very common in the studied population and had different age distributions, possibly indicating differences in transmission routes, immunity, and/or other host factors for these two species

In the present study, the commonest clinical presentation or complaint among studied cases was abdominal pain or discomfort (78%). This was in concordance with Ibrahium (2011) who reported that abdominal colic was the commonest complaint among rural primary school pupils in Minia Governorate, Egypt. Also, Swamy *et al*. (2013) studied soil-transmitted helminths in children in India and reported that maximum children presented with abdominal pain. On the other hand, Eltayeb *et al*. (2016) in Sudan concluded that the majority of schoolchildren with parasitosis suffered from iron deficiency anemia, followed by haemolytic, macrocytic and sickle cell anemia

In the present study, the semiformed consistency (77%) was the commonest among stool samples followed by formed ones (17.08), which agreed with Abou-Madyan *et al.* (2004) in schoolchildren in rural area n Fayoum Governorate

Thus, direct wet mount was simple, easy and rapid but it dried up quickly after preparation and was of very low sensitivity; about three times less sensitive than the formol ether concentration method. Kato-Katz thick smear was of low sensitivity, being about twice as less sensitive as the formol ether concentration method. High risk of infection because of handling fresh stool is another drawback. Also, spontaneous sedimentation in tube technique was simple and inexpensive. The sensitivity was (81.3%) being compared to FEC. It is considered a valid technique. The formol ether concentration method was considered the gold standard. It allowed transportation and storage of formalin preserved feces with less risk of infection. However, it couldn't be performed in the field as it needs centrifuge and electricity.

Conclusion

The outcome data showed that formol ether concentration method was superior to spontaneous sedimentation in tube technique,

Kato-Katz thick smear and direct wet mount methods for routine diagnosis of fecal-orally trans-mitted helminthes and other geohelminthes.

Recommendations

The negative stool samples by direct wet mount method should be re-examined by the formol ether concentration technique. Multiple stool samples from an individual is recommended to reveal true prevalence. Availability of adequate health care and routine medical examination and laboratory investigations for children is a must. Stool culture is recommended in suspected *S. stercoralis*.

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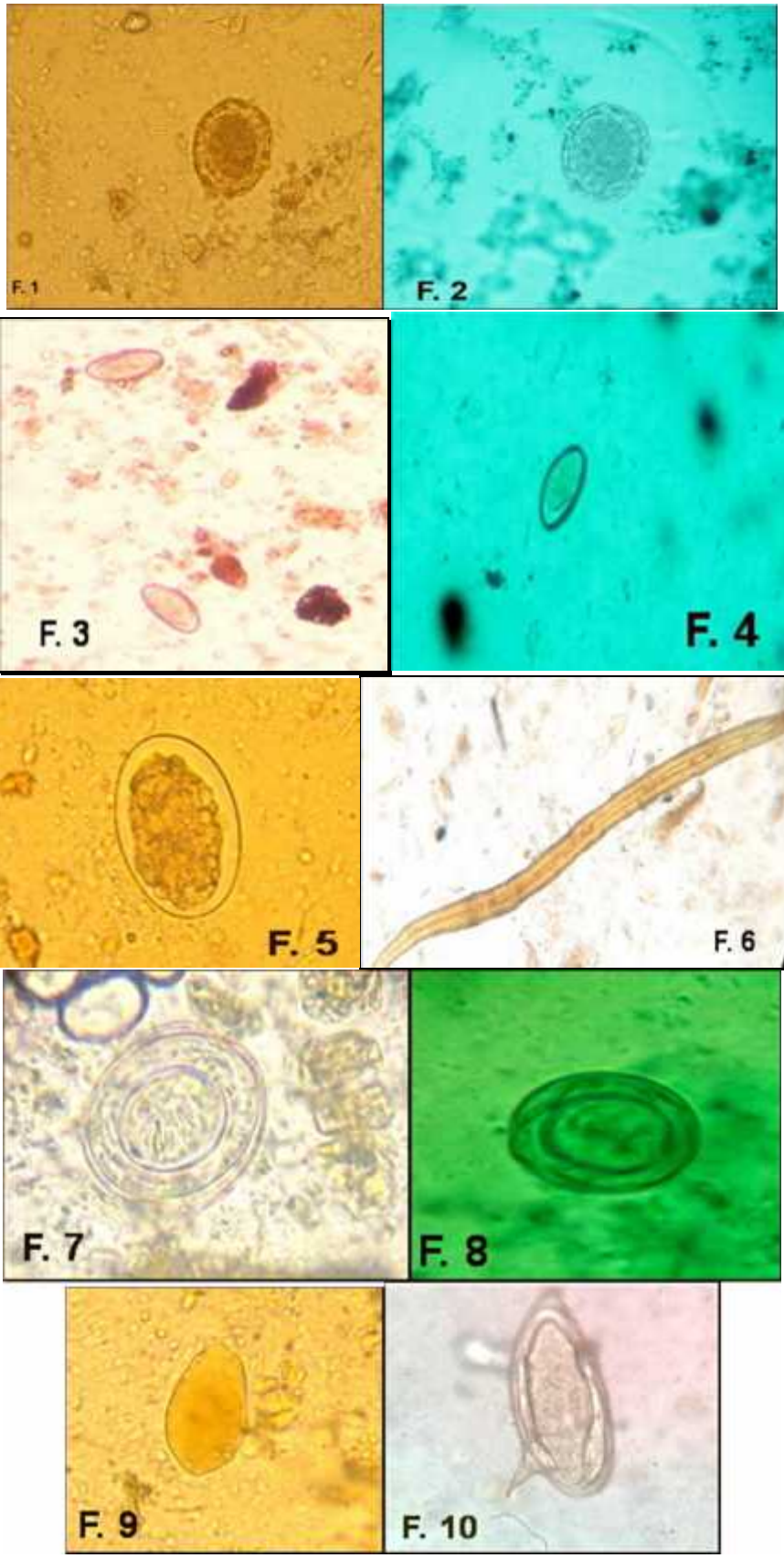
Explanation of figures

Fig. 1: *A. lumbricoides* egg by DWM (X100).
Fig. 2: *A. lumbricoides* egg by Kato-Katz thick smear (X100).
Fig. 3: *E. vermicularis* egg by DWM (X100).
Fig. 4: *E. vermicularis* egg by Kato-Katz thick smear (X100).
Fig. 5: *A.duodenale* egg (X100).
Fig. 6: *S. stercoralis* larva (X400).
Fig. 7: *H. nana* egg by DWM (X400).
Fig. 8: *H. nana* egg by Kato-Katz thick smear (X400).
Fig. 9: *H. heterophyes* egg (X400).
Fig. 10: *S. mansoni* egg (X100).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