

PARASITIC STAGES ISOLATION FROM SOIL SAMPLES OF KIRKUK TECHNICAL COLLEGE

Hiro M. Obaid

Environmental and Pollution Engineering Techniques
Department, Kirkuk Technical College, Northern
Technical University, Iraq. dr.salai@ntu.edu.iq

Introduction:

Soil transmitted parasitic infection constitute one of the major human problems, it infects vast number of populations. Worldwide, it was evaluated in 2010, that 438.9 million individuals were diseased with hookworm, 819.0 million with *Ascaris lumbricoides* and 464.6 million with *Trichuris trichiura*, and that the great amount (67%) of soil transmitted infections happened in Asian countries [1]. These infections have a significant impact on human health and wealth. Many infectious diseases agents reside in soil as it represents a good environment and offer shelter and nutrient source for these organisms, including parasites [2]. Some of these parasites spend their whole life in soil and some spends parts or stages in soil. It seems that most of soil resident parasites infect human and animals [3]. *Storonyloides stircoralis*, one of soil helminthes can have a free life cycle in soil and opportunely may transform to a serious parasite of human [4]. Human hookworm filariform infective larva live in soil surface layer. Many other dangerous helminthic and protozoan stages can be found in soil such as: *A. lumbricoides*, *T. trichuras* *Taenia sp*, *Echinococcus granulosus* [5]. *Entamoeba*, and *Giardia*, cysts, *Cyclospora* and *Cryptosporidium* oocyst [6, 7]. People works or with contact with soil are more at risk for getting soil parasite [8]. The principal pathway for getting soil parasites is through ingestion or skin contact [5, 6]. Several studies have been carried out on soil parasites. In a study of Kirkuk city, using zinc sulphate flotation methods, 78.6% of soil samples were positive for helminthic eggs in Kirkuk public parks and playgrounds [9]. Several other diagnostic methods were used for isolation of *Ascaris*, *Storonyloides*, *Toxocara* in Baghdad city [10], Nematode and Cestode eggs were identified form soil samples of Erbil city [11], *Eimeria* oocysts was isolated form soil samples using flotation method [12]. Evidence indicated the presence of geohelminthic egg and protozoan oocysts in Tehran public places [13]. Huge number of helminthic eggs were documented in soil samples of Kenya and Bangladesh [14]. 31% of examined samples were positive for soil helminthes eggs in certain urban and rural parts in Phillipine [15]. The aim of this investigation was, to determine the dominance of parasitic stages (eggs, larvae, cysts, oocysts) in soil samples from various sites of Kirkuk Technical College.

Materials and methods:

1- Soil samples collection: During the period of July to December 2017, 110 soil samples were collected from various sites of Kirkuk Technical College by simple random selection. Initially, the college was geographically divided into five regions : north, south, east, west and center. 22

samples were collected from each region. In each collection approximately 100 g was gathered from 3 cm ground depth [16]. All samples were moist. The samples were scanned by direct wet mount, sedimentation, floatation, Modified acid - fast stain and Baermann method.

2- PH and moisture measuring: In order to measure the soil samples PH, a PH meter apparatus was pleased for 2 minutes in a soil water mixture, prepared from 2gm soil and 1ml water. The stated PH then was recorded. For soil moisture, the moist soil was first weighted, then dried in an oven at 125°C for 24 hours. The moisture level was estimated using this equation [15].

$$\text{Moisture level} = \frac{\text{soil moist weight} - \text{soil dry weight}}{\text{soil dry weight}} \times 100$$

3- Microscopic examination (direct wet mount normal saline): After a small amount of the soil was emulsified with normal saline, filtered with gauze to remove any big elements. Mixed well with wooden stick. A drop was add to a clean slide. The specimens were examined first with the low power objective for helminthic ova and larva then with high power objective for protozoan stages [9].

4- Sedimentation method (formalin-ether) : For formal-ether concentration method, 1-2 g of soil was mixed in 10 ml distilled water and filtered. The filtrate was centrifuged at 2500 rpm for 2 minutes. Supernatant was discarded and the sediment was re-suspended in 7 ml of formal saline (10%), 3 ml of ether was added, the tube was stoppered and was and mixed carefully. The mixture was centrifuged at 2500 rpm for 2 minutes. The three layers of ether, debris and formal saline were discarded. The sediment was re-suspended and examined under microscope [4].

5-Saturated salt floatation technique: About 1g of soil was shaken up in 2 ml of sodium chloride saturated salt solution with a specific gravity of 1.2. The tube was then completed to the edge with salt solution. A clean cover slide without any intervening air bubbles was placed on the top of the tube. After 20-30 minutes, the cover was removed, put on a slide and microscopically examined [4].

6- Modified kinyoun's acid - fast stain: A thin smear of moist soil sample was done on a clean microscope slide, air dried. The smear was swamped with Kinyoun's carbol fuchsin for 5 minutes then rinsed for 3-5 seconds with 50% ethanol then with water. The samples were decolorized with 1% sulfuric acid for 2 min. The acid was removed with water. Counter stained with malachite green for 1 min., washed with water. Air dried and microscopically examined [13].

7- Baermann method: About half to one cup of recently collected soil was spread cautiously on a gauze layer placed on a mesh which was set on a class funnel as shown in figure 1(I). Tap water was added to the funnel until the water surface is scarcely touched the mesh. The fixed clamp at the funnel end was secured. Carefully additional amount of water was added until the water surface was almost above the top of the tissue. The apparatus was placed overnight at room temperature. The clamp was cautiously released to collect about 5 ml of solution in a dish. The solution was viewed with magnifying lens then microscopically examined [17].

Results:

From each location of the five sites chosen of Kirkuk Technical College, 22 soil samples were collected. As it

shown in table 1, 23.63% of examined samples were positive for helminthic and protozoan parasites. The East part was the

most contaminated site of the college with a rate of 45.45% while the South part was the lowest with a rate of 4.54%.

Table 1: Intensity of soil parasites according to locations

Sites	Screened samples	+ve samples	%	-ve samples	%
Center	22	2	9.09	20	90.90
West	22	5	22.7	17	77.27
South	22	1	4.54	21	95.45
East	22	10	45.45	12	54.54
North	22	8	36.36	14	63.63
Total	110	26	23.63	84	76.36

Cryptosporidium parvum oocysts. *Toxocara sp.* had the highest level of occurrence, followed by *S. stercoralis*, the lowest was for *A. lumbricoides* and *E. histolytica*. Figure 1 shows identified parasitic stages.

Table 2 shows the frequency of isolated soil parasites. The overall isolated parasites were 8 genera they included: *Toxocara sp.*, *Trichuris trichiura*, *Hymenolepis nana*, *Ascaris lumbricoides* eggs, *Strongyloides stercoralis* larva, *Giardia lamblia* and *Entamoeba histolytica* cysts and

Table 2: Isolated parasites from different locations

Sites	Parasites species															
	Helminths						Protozoa									
	<i>A. lumbricoides</i>		<i>S. stercoralis</i>		<i>T. trichiura</i>		<i>Toxocara sp.</i>		<i>H. nana</i>		<i>G. lamblia</i>		<i>E. histolytica</i>		<i>C. parvum</i>	
NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	NO.	%	
C	0	0	1	5	0	0	1	5	0	0	0	0	0	0	0	
W	0	0	1	5	0	0	1	5	0	0	0	0	0	1	5	
S	0	0	1	5	0	0	0	0	0	0	0	0	0	0	0	
E	1	5	1	5	1	5	3	13.6	1	5	2	9	1	5	1	5
N	0	0	2	9	1	5	3	13.6	1	5	2	9	0	0	0	0
Total	1	0.9	6	5.5	2	1.8	8	7.3	2	1.8	4	3.6	1	0.9	2	1.8

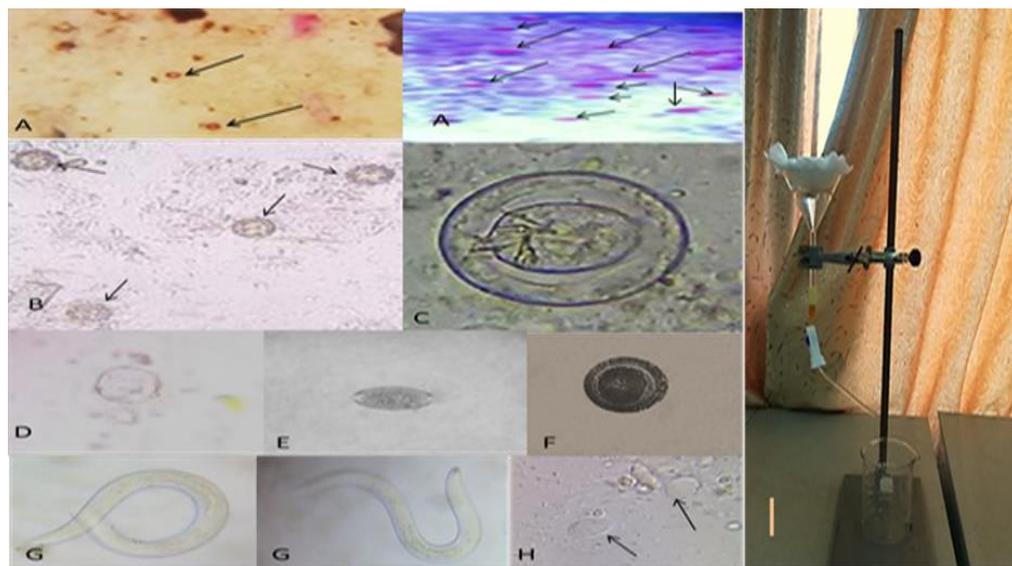


Figure 1: Soil isolated parasitic stages, Baermann apparatus . A= *Cryptosporidium* oocysts, B= *Ascaris* eggs, C= *H.nana* egg, D= *E. histolytica* cyst, E= *T. trichiura* egg, F= *Toxocara* egg, G= *Strongyloides* larva, H= *Giardia* cyst, I= Baermann apparatus.

Table 3 illustrates that concentration methods (sedimentation and floatation) were more effective in detecting soil parasites in comparison with the other utilized techniques. Some parasites were identified efficiently only by specialized

methods such as modified acid fast stain or Baermann method. Nearly equal parasites frequency was noted in both of Autumn (57.69%) and Winter (42.30%) seasons.

Table 3 : parasites frequency according to used techniques and seasons

Parasites	Number of +ve samples using different techniques					Seasons		
	Direct wet mount	Sedimentation	Floatation	Modified acid fast stain	Baermann method	Autumn Frequency (%)	Winter Frequency (%)	Total frequency (%)
<i>A. lumbricoides</i>	0	1	0	0	0	1(3.84)	0(0.00)	1(3.84)
<i>S. stercoralis</i>	2	0	0	0	4	4(15.38)	2(7.69)	6(23.07)
<i>T. trichiura</i>	0	2	0	0	0	2(7.69)	0(0.00)	2(7.69)
<i>Toxocara sp.</i>	3	5	8	0	0	6(23.07)	2(7.69)	8(30.76)
<i>H. nana</i>	0	2	0	0	0	1(3.84)	1(3.84)	2(7.69)
<i>G. lamblia</i>	2	4	3	0	0	1(3.84)	3(11.53)	4(15.38)
<i>E histolytica</i>	1	1	0	0	0	0(0.00)	1(3.84)	1(3.84)
<i>C. parvum</i>	0	0	0	2	0	0(0.00)	2(7.69)	2(7.69)
Total	8	15	11	2	4	15(57.69)	11(42.30)	26(23.63)

Parasitic stages were more found in acidic soil than in alkaline samples. Soil moisture had effected the parasites occurrence, high number of positive samples were detected

in moderate level of moisture (16-45) than in high or low levels, table 4.

Table 4: Frequency of parasites according to soil PH and moisture

PH	No. +ve samples	Moisture	No. +ve samples
6.3-6.5	10	5.20-15	2
6.6-6.8	8	16-30	9
7-7.2	5	31-45	8
7.3-7.5	1	46-61	5
7.6-7.8	2	62-85	2

Discussion:

The present study revealed that, the soil samples collected from Kirkuk Technical College were contaminated with high level of parasite stages (23.63%), this represents a warning of soil contamination with human and animals' feces. This contamination level was much less than that found in Kirkuk parks and ply yards (78.6%) [9], and that found in Erbil parks (91.6%) [11]. This is down to the differences between the chosen area in each study. The public parks and ply yards are thought to be more contaminated due to their large sizes and opening hours to public use during day and night. Both helminthic and protozoan stages were found contaminating soil samples in this study, the highest contamination level

was for *Toxocara sp.* eggs with rate of 7.3% followed by *S. stercoralis* larva with rate of 5.5%. Our result was conforming with that investigation on humans infection with toxocariasis in Mosul whom reported a rate of 7.3% among healthy individuals [18]. Moreover the results of all tested soil sample from different regions had virtually revealed highest occurrence of *Toxocara* [19-21], this is most probably due to uncontrolled or stray dogs and cats infected with *Toxocara*, in addition to the numerous means of the worm transmission which may increase its prevalence. Furthermore the eggs of *Toxocara* can remain alive for years in outside environments. Likewise *Strongyloides stercoralis* continue dormant in moist soil or stay a life on vegetation's leaf near to the soil surface [4]. Other researchers had

recorded the same or other helminthic or protozoan parasites similar to our study, in a study on helminthes contamination of Kirkuk parks and ply yards, they recorded *A. lumbricoides* (19.4%), *H. diminuta* (16.7%), *A. duodenale* (13.9%), *T. saginata* (11.1%), *T. trichiura* (8.3%) and *Trichostrongylus spp.* (8.3%) [9]. Helminthic eggs and larva or adult worms and protozoan cysts or oocysts had been isolated from soil samples in different pleases such as Baghdad, Erbil, Iran, Bangladesh, Philippine, Nepal, and Nigeria [10-15, 22-23]. The isolated parasitic stages had slightly differed between these studies, this could be because of differences in the epidemiological factors effecting the distribution and incidence of these parasites in each region. Concentration method was more efficient in detecting most of isolated parasites in current study, some other used techniques were specific like modified acid fast and Baermann methods. Similarly, other studies found sedimentation and flotation to be more capable in isolating some parasites [10, 13-15]. This is because these methods will concentrate the parasites in the microscopic field due to utilization of solutions with low or high specific gravity that make the parasites float or sediment [4]. This study had found higher incidence of parasites in Autumn than in Winter seasons, Autumn season recovered 15 (57.69%), while winter season recovered only 11 (42.30%). Identical results were found by [22,23] whom revealed higher prevalence in dry season than in moist season. In addition, parasitic stages were more frequent in acidic and moderate moist soil samples, which was in line with Paller *et. al* [15] whom fond identical results. This can be attributed to the suitable environmental and climate conditions, with moderate temperature and sufficient soil humidity during Autumn unlike Winter season in which high rates of rainfall may wash of these parasitic stages from the soil or Summer season in which parasites may be destroyed by high temperature and direct sunlight [10].

PARASITIC STAGES ISOLATION FROM SOIL SAMPLES OF KIRKUK TECHNICAL COLLEGE Hiro M. Obaid.

Introduction: Soil is an influential source of human diseases, including parasitic infections. Parasitic diseases represent a potential risk factor on human health and his belongings. This study aimed to evaluate soil contamination level with parasitic stages of soil samples from Kirkuk Technical College. **Methods:** 110 soil samples were collected from various sites of Kirkuk Technical College by simple random selection. The samples were scanned by direct wet mount, sedimentation, floatation, Modified acid-fast stain and Baermann method. **Results:** The overall rate of parasitic contamination was 23.63%. The most prevalent parasite stage was *Toxocara sp.* eggs with rate of 7.3%, followed by *S. stercoralis* larva with rate of 5.5%. Concentration technique was more effective in detecting soil parasites comparing with the other used methods. Parasites prevalence was approximately similar in both Autumn and Winter months. Soil parasites were more existed in acidic and moist soil samples. **Conclusions:** Different helminthic and protozoan stages are

contaminating soil samples of Kirkuk Technical College; these stages can effectively be detected by concentration method. **Recommendations:** Efforts are better to be directed toward eliminating these parasites through using appropriate ways for waste handling and controlling stray dogs, cats and rodents in the college. More investigations are required on parasitic prevalence among this college staff and students.

Key words: Parasitic stages, Isolation, Soil samples, Kirkuk.

References:

- 1.Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. Scie Worl J.2014, Article ID 386232, 6 pages.
- 2.Mandarino-Pereira A, de Souza FS, Lopes CWG, Pereira MJS. Prevalence of parasites in soil and dog feces according to diagnostic tests. Vet Parasitol.2010;170(1-2):176-18.
- 3.de Silva NR, Brooker S, Hotez PJ, Montresor A, Engels D, Savioli L. Soil-transmitted helminth infections: updating the global picture. Trends Parasitol. 2003;19(12):547-55.
- 4.Arora DR, Arora B. Medical Parasitology. 2nd ed. New Delhi. Bangalore India. CBS Publishers and Distributors.2009; 220-21.
- 5.Waenlor W, Wiwanitkit V. Soil examination for soil-transmitted parasite: importance and experience from Thailand. J Ped Inf Dis.2007;2(1):11-13.
- 6.Davies CM, Altavilla N, Krogh M, Ferguson CM, Deere DA, Ashbolt NJ. Environmental inactivation of *Cryptosporidium* oocysts in catchment soils. J App Microbio.2005;98(2):308-17.
- 7.Kola BS, Racheal AM, Elizabeth AA, Ojo S, Nimat A. Soil-transmitted helminth infections among school children in rural communities of Moro Local Government Area, Kwara State, Nigeria. African J Microbio.2013;7(45):5148-53.
- 8.Ross AGP, Olveda RM, McManusc DP, Harn DA, Chye D, Li Y, et al. Risk factors for human helminthiases in rural Philippines. Inter J Infec Dis.2017;54:150-55.
- 9.Taher HM. Soil contamination with intestinal parasites eggs in public parks and playgrounds in Kirkuk city. Tikrit J Pure Sci.2017;22(6):52-5.
- 10.AI-Khamesi MB. Isolation and diagnosis of parasites from different soils in Baghdad City. J Al-Nahrain Univ.2014;17(3):155-61.
- 11.Nooraldeen K. Contamination of public squares and parks with parasites in Erbil city, Iraq. Ann Agric Enviro Med.2015; 22(3):418-20.
- 12.Assen B, Epik T. Isolation of *Eimeria* oocysts from soil samples: a simple method described in detail. J Agric Sci.2014;2(25):77-81.
- 13.Tavalla M, Oormazdi H, Akhlaghi L, Razmjou E, Moradi M L, Shojaee S. et al. Prevalence of parasites in soil samples in Tehran public places. African J Biotech. 2012;11(20):4575-78.
- 14.Steinbaum L, Kwong LH, Ercumen A, Negash MS, Lovely AJ, Njenga SM. et al. Detecting and enumerating soil-transmitted helminth eggs in soil: New method

development and results from field testing in Kenya and Bangladesh. PLoS Negl Trop Dis.2017;11(4):1-15.

15.Paller VGV, De Chavez ERC. *Toxocara* (Nematoda: Ascaridida) and other soil-transmitted helminth eggs contaminating soils in selected urban and rural areas in the Philippines. Scie Worl J.2014;386232. 6 pages.

16.Nkouayep VR, Tchakounté BN, Poné JW. Profile of geohelminth eggs, cysts, and oocysts of protozoans contaminating the soils of Ten primary schools in Dschang, West Cameroon. J Parasitol Res.2017;1534675, 6 pages.

17.Barrière A Félix MA. Isolation of *C. elegans* and related nematodes. Worm Book, ed. The *C. elegans* Research Community, Worm Book, 2006. <http://www.wormbook.org>.

18.Woodruff AW, Waston J, Shikara L, Al-Azzi NSH, Al-Hadithi TS, Al-Adhami SBH.et al. *Toxocara* ova in soil in the Mosul district, Iraq and their relevance to public health measures in the Middle East. Ann Trop Med parasite.1981;75(5):555-57.

19.Maleki B, Seyyed TS J, Tahvildar F, Khorshidi A. Soil contamination of public places with *Toxocara spp.* Egg in Kermanshah, Iran, in 2014. Novel Biomed.2016;4(3):105-9.

20.Traversa D, Di Regalbono AF, Di Cesare A, La Torre F, Drake J, Pietrobelli M. Environmental contamination by canine geohelminths. Para Vect.2014;7:67.

21.Tudor P. Soil contamination with canine intestinal parasites eggs in the parks and shelter dogs from Bucharest area. Agricu Agricu Scie Procedia 6.2015;387-91.

22.Rai SK, Uga S, Ono K, Rai G, Matsumura T. Contamination of soil with helminth parasite eggs in Nepal. Southeast Asian J Trop Med Public Health.2000;31(2):388-93.

23.Nwoke EU, Ibiam GA, Odikamnoru OO, Umah OV, Ariom OT, Orji I. Examination of soil samples for the incidence of geohelminth parasites in Ebonyi North-Central area of Ebonyi State, South-East of Nigeria. Arch Appl Sci Res.2013; 5(6):41-8.