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Prevalence of *Cyclospora cayetanensis* and Other Intestinal Parasites in Soil Samples Collected from Kirkuk Province

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A B S T R A C T

Environmental contaminations with pathogens, especially soil and water had a great role in transmitting microorganisms, particularly in recent decade *Cyclospora cayetanensis* that cause several outbreaks of diarrhea in several countries in tropic and sub-tropical countries. The aim of current study was to determine the prevalence of Cyclospora in three types of soil samples collected from different regions in rural and urban area within a period from 1st of October 2014 to 31st of May 2015 within which a total of 245 soil samples were examined macroscopically and microscopically using double wet preparations of 0.85 % of NaCl solution and 1 % of Lugols iodine stain and flotation technique (zinc sulphate solution) for detecting parasitic stages with an emphasize on *Cyclospora oocysts*. The overall rate of the parasitic stages was 242/98.77, this rate was involved 62.03% of protozoan parasites versus to 36.74 % for helminthes, $P<0.05$. Common parasites involve 11 protozoa and 9 species of helminthes, within which, high rates were exerted as *Giardia lamblia*, *Cryptosopridium parvum*, *Trichomonas hominis* and *Cyclospora cayetanensis*, *Strongyloides stercoralis*, *Ascaris lumbricoides*, *Ancylostoma* species. The rates were: 20%, 14.28%, 13.46%, 11.02%, 16.79%, 6.33% and 6.33% respectively. For the first time 5/2.04% of Osterusovis and 1/0.40% of *Physaloptera preaputialis* were recorded in Kirkuk city soil. Statistically, direct double wet preparation technique reveal high efficacy in demonstrating of all parasites than using flotation technique ($ZnSO_4$), controversy to none statistical differences between two employed technique in regard of helminthes. Incidence of parasite were high in clay soil samples 35.91% and 22.85% for protozoan parasites and helminthes, $P<0.05$, followed by 15.10%, 4.08% in mixed soil samples for both groups respectively, whereas in sandy soil the rates included 12.24% and 8.91% for protozoan and helminthic parasites, respectively. *Cyclospora cayetanensis* incidence according to type of soil samples was high 6.12 % in clay soil compare to 2.85 % in mixed soil and 2.07 % in sandy soil, $P<0.05$. Protozoan parasites found in high rate 40.81% in urban area via which 7.34 % was for *Cyclospora cayetanensis* versus to 21.22 % of overall rate protozoan parasites in rural area. Also *Cyclospora cayetanensis* rate in rural area was 4.08 %. Soil samples in Kirkuk Province were highly contaminated with parasites particularly clay soil type. *Cyclospora cayetanensis* oocysts act as one causes of soil contamination in Kirkuk province. Direct double wet preparations of soil samples were with high efficacy in demonstrating protozoan parasites particularly *Cyclospora cayetanensis* than flotation technique.

Introduction

Soil in anywhere is a part from any community; its neatness can provide good health for living creatures on or in it, otherwise diseases are raised to harm human being (Gugino *et al.,* 2009). Soil contamination with microorganisms and free living of parasitic worms in soil, such as *Strongyloides stercoralis* and hook worms (*Ancylostoma, Necator* species) was studied in detail by interested authors (Mustafa, 2013). While regarding protozoan parasite distribution in soil samples was poorly studied, particularly *Cyclospora* species including *C. cayetanensis*. This parasite is an important emerging cause of diarrhea worldwide that leads to significant morbidity and mortality (Chacín-Bonilla, 2010). In developing countries, the parasite has been associated with diarrhea in health center populations. However, in communities based studies, infection rates up to 41.6% have been noted and most of the cases are asymptomatic (Eberhard *et al.,* 1999). Cyclosporiasis is now pretty common infection rates of 5.3% in diarrheic children, 6.8-9.8% in AIDS patients, and 6.1–11.9% in communities has been found. In the developing world, the infection has been associated with variables related to water, foods, and animals, and contact with soil (Mansfield and Gajadhar, 2004). The coccidium has been found in water, vegetables, bivalves, and animals. However, the role that the latter may play in the epidemiology of cyclosporiasis remains controversial (Chacín-Bonilla, 2008).

Cyclospora was first reported in Papua New Guinea in 1979 as an oocystlike body found in 3 patients with intestinal infections. From 1986 to 1991, several reports described diarrhea associated with a large " *Cryptosporidium* " or cyanobacterium like bodies in both immune-competent and immunosuppressed patients from North, Central, and South America; the Caribbean; Nepal; India; and Southeast Asia (Lopez *et al.,* 2003). In 1993, in Lima, Peru, Ortega et al characterized and clarified remaining taxonomic issues for *Cyclospora cayetanensis* (Ortega *et al.,* 1997). The laboratory diagnosis of newly recognized infectious agents, such as *Cyclospora cayetanensis*, is frequently problematic because appropriate diagnostic techniques and algorithms are not available. The methods currently available for diagnosis of *Cyclospora* are described and compared, including concentration procedures, examination of wet preparations, various staining techniques, and the use of molecular-based assays. Because of the auto-fluorescent properties of the oocysts, particular attention is drawn to the role of fluorescent microscopy in providing a rapid, inexpensive, and sensitive technique for diagnosis of *Cyclospora* infections in stool samples (Eberhard *et al.,* 1997).

The first record of *Cyclospora* in Arab country was in Basra –Iraq in 1999 in three diarrheic cases recorded by Ali and Mahdi (1999). Followed by two other studies in children in Baghdad and Salahadeen by Al-Aakely in 2007 and Al-Samarria in 2008 whom they record 1.6% and 1.2 % of *Cyclospora oocysts* in stool samples respectively. While the following rates: 6 %, 4.75 and 5.6 % were recorded in Jordon, Saudi Arabia, and in Egypt by Nimri (2003), Mansfield and Gagadhar (2004) and El-Karamy *et al.* (2005) respectively. According to available literatures, scientific studies regarding parasites and our information; studies concerning *Cyclospora cayetanensis* distribution in soil samples are not present in Iraq and to emphasize on role of direct microscopy and flotation techniques in detecting the oocysts of this parasite. So this study was conducted at to

perform role of soil samples in distributing of this parasite in Kirkuk Province using direct double wet preparations and flotation ($ZnSO_4$) techniques.

Materials and Methods

Time and location: From 1st of October 2014 to 31th of May of 2015, field study was conducted in laboratory department of dentistry College-Kirkuk University, and in Ibn-Nafies private medical laboratory.

Soil Samples collection: A special nylon packs of about one kilogram capacity were purchased from local markets in Kirkuk city to collect soil samples. Prior to collection each pack was labeled with required essential information. Kirkuk Province was divided into two regions rural and urban areas, a total of 75 soil samples were collected from northern rural area of Kirkuk Province. While 170 samples were collected from urban area that covered all districts from 4 common directions. The apical part of each pack was fitted tight with rubber and brought to laboratory as soon as collected. Eight samples from urban area and 5 soil samples from rural area were ignored due to texture changed with in the packs. Daily 2-3 samples were collected with average of 12 samples per week without adding any preservatives.

Laboratory processing

Sample sieving: Using a metal sieve with small pores about 2x3 mm, each specimen was sieved to expel out large particles and plant parts. By using naked eyes the upper part was watched for Cestoda worm segments and to adult nematodes including *Ascaris* adult worm, earth worm and other foreign subjects.

Macroscopic examination: by using the aid of hand lens piece and light source, the sieved filtrate soil was observed for adult worms and larval stages of hookworms. Soil specimen color and textures involves clay soil, sandy soil and mixture soil were adjusted.

Microscopic examinations: Prior to microscopic slide preparations, a special procedure was suggested and applied by team work on each soil specimen. Which include weighing of 250 sieved soils by electrical balance, then after about 500 ml of clear unused distilled water (D.W) was transferred in to clean plastic container unused previously. Both sieved soil and D.W were mixed thoroughly by large clean spoon. The mixture was left for about 30 minutes. Initial microscopic examination involves direct preparing of double wet mount of 0.85 % NaCl, 1 % of Lugols iodine and flotation ($ZnSO_4$ solution 33 %). The mixture was covered with lid and fitted tight with tape to avoid escapes of worms during overnight incubation if the specimen contains helminthes. While second microscopic examination was done in the second day, also involves the same techniques as used in initial exam. After that the specimen was covered with the lid and tape again and left at optimum laboratory temperature.

Every day the incubated specimen was watched and extra of D.W, was added to avoid dryness due to consumption by soil biota, fauna or due incubation. Final microscopic examination was done on the 7 days of incubation which involve the techniques that applied on in initial exam. Direct double wet preparations and flotation ($ZnSO_4$) techniques were done similar to that routinely be applied on stool samples in medical laboratories and according to standard stool examination procedure for intestinal parasites described by W.H.O. in 1991. All data were accumulated, arranged

in tables and checked statistically for detecting significant difference between current study parameters using Chi-square and t-student tests at probability of 0.05 and 0.001.

Result and Discussion

From the examining of 245 soil samples collected from different regions in Kirkuk Province 242/98.77 % were contaminated with parasites. This rate was contribute 62.03 % of protozoan parasites followed by 36.74 % of parasitic helminthes, P<005. Statistically direct double wet preparations technique reveal high overall rate 58.77% compare to 40 % by using flotation technique (33% of $ZnSO_4$ solution). Efficacy of double wet preparation for detecting protozoan parasite was higher than detecting of helminthes, the rates were 39.18%, 19.59% respectively, P<0.05. Controversy to no significant differences between protozoan and helminthes rate using flotation technique, P>0.05. Collectively relationship between laboratory two employ techniques and existence of parasites during soil examination was significant (P <0.05) (Table 1).

Table.1 Distribution of parasitic infections in soil samples using direct and floatation technique

Types of parasites	Direct wet preparation Positive		Flotation technique Positive		Total Positive	
	No.	%	No.	%	No	%
Protozoa	96	39.18	56	22.85	155	62.03 *
Helminthes	48	19.59	42	17.15	90	36.74
Total	144	58.77 **	98	40.00	242	98.77

*,** P<0.05. Total examined soil samples =245

Table.2 Positive percentages of common protozoan parasites in soil samples according to two lab methods

Parasites /Protozoa	Direct wet preparation Positive		Flotation technique Positive		Total Positive	
	No.	%	No.	%	No.	%
Cyclosporacayetanensis	14	5.71	13	5.30	27	11.02
Cryptosporidium parvum	17	6.93	18	7.34	35	14.28
Giardia lamblia	28	11.42	21	8.57	49	20.00 *
Blastocyst hominis	0.00	0.00	1	0.40	1	0.40
Trichomonas hominis	29	11.83	4	1.62	33	13.46
Balantidium coli	2	0.81	0	0.00	2	0.81
Toxocara canis	2	0.81	9	3.67	2	0.81
Microspora spp	2	0.81	2	0.81	4	1.62
Isospora spp	1	0.40	0	0.00	1	0.40
Giardia canis	1	0.40	0	0.00	1	0.40
Retromonas intestinalis	1	0.40	0	0.00	1	0.40
Total of protozoa	97	39.58 *	68	27.75	164	67.37

Table 2 was summarizing the common protozoan parasites found in current study. High rate 20% was *Giardia lamblia*, followed by 14.28%, 13.46 and 11.02 % for

Cryptosporidium parvum, Trichomonas hominis and Cyclospora cayetanensis respectively versus to 0.40 % for each of the following parasites: *Blastocystis hominis, Isospora* spp and *Giardia canis.* Relationship between protozoan parasites distribution and type of laboratory technique was significant (P<0.05).

Total examined soil samples =245 *P<0.05 some specimen exert co-infection so, the variance in total number and positive percentage compare to that in table 1.

Soil from Kirkuk Province was highly contaminated with *Strongyloides* stercoralis 16.79 %, followed by *Ascaris lumbricoides* and *Ancylostoma* species for each one 6.33%.whereas lowest rates involve Eggs of *Echinococcus granulosus* and *Physaloptera prepuitialis* for each 0.40 % equally, P<0.05.Statistically relationship between helminthes distribution in regard of laboratory techniques employed was not significant (P>0.05) (Table 3).

Total examined soil samples =245 *P<0.05 some specimen exert co-infection so, the variance in total number and positive percentage compare to that in table 1.

Soil samples in current study were sandy, clay or mixed soil were examined for protozoan parasites using both direct double wet preparations and flotation techniques, vis. which's high rate of protozoan parasites 35.91 % was recorded in clay soil. While in mixed and sandy soil samples the rates were 15.10% and 12.24% respectively, P, 0.05.*Giardia lamblia* was highly found 11.83 % in clay soil samples, followed by8.57%,6.98 % and 6.12% for *Cryptosporidium parvum, Trichomonas hominis* and *Cyclospora cayetanesis* respectively compare to lowest rates with other protozoan parasites found in current study (P<0.05).

Table.3 Frequency of parasitic helminthes in soil samples according to two lab methods

Parasites/Helminthes	Direct wet preparation positive		Flotation technique positive		Total positive	
	No.	%	No.	%	No.	%
Ascaris lumbricoides	8	3.26	8	3.26	16	6.53
Ancylostoma spp	6	2.44	10	4.08	16	6.33
Strongyloides stercoralis	20	8.16	21	8.57	41	16.79 *
Lumbricus terrestris	3	1.22	0	0.00	3	1.22
Taenia spp	1	0.40	1	0.40	2	0.81
Enterobius vermicularis	3	1.22	1	0.40	4	1.63
Osterusovis	4	1.63	1	0.40	5	2.04
Physaloptera prepuitalis	1	0.40	0	0.00	1	0.40
Echinocaccus spp	1	0.40	0	0.00	1	0.40
Total of helminthes	47	19.19	42	17.14	90	36.34

Table.4 Distribution of protozoan parasites according to types of collected soil samples

Type of parasites	Mixed soil		Sandy soil		Clay soil	
	N	%	N	%	N	%
Protozoa						
Cyclospora cayetanesis	7	2.85	5	2.07	15	6.12
Cryptosporidium parvum	6	1.22	8	3.26	21	8.57
Giardia lamblia	12	4.89	8	3.26	29	11.83*
Blastocyst hominis	0	0.00	0	0.05	1	0.40
Trichomonas hominis	8	3.26	8	3.26	17	6.98
Balantidium coli	2	0.81	0	0.00	0	0.00
Toxocara canis	1	0.40	0	0.00	1	0.40
Microspora species	0	0.00	1	0.40	3	1.22
Isospora species	0	0.00	0	0.00	1	0.40
Retromonas intestinalis	0	0.00	0	0.00	1	0.40
Giardia canis	1	0.40	0	0.00	1	0.40
Total of protozoa	37	15.10	30	12.24	88	35.91*

*P<0.05

Table.5 Helminthes frequency in relation to types collected soil

Helminthes	Mixed soil		Sandy soil		Clay soil **	
	No	%	No	%	No	%
Ascaris lumbricoides	1	0.40	5	2.5	10	5
Ancylostoma spp	0	0.00	5	2.5	11	5.5
Strongyloides stercoralis	9	4.5	10	5	22	11**
Lumbricus terrestris	0	0	0	0	3	1.5
Taenia spp	0	0	0	0	2	1
Enterobius vermicularis	0	0	1	0.5	3	1.5
Osterusovis	0	0	0	0	4	2
Physoleptra preputalis	0		0	0	1	0.5
Echinocaccus granulosus	0	0	1	0.5	0	0
Total	10	4.08	22	8.91	56	22.85

*,** P<0.005.

Table 5 is clarifying role of types of soil samples in regard of parasitic helminthes, as it was shown in table 4; clay type soil reveal high rate of helminthic parasites 22.85 %,with high dominance rate 11% of *Strongyloides stercoralis* compare to 5% and 4.5 % for the same parasite in sandy and mixed soil samples respectively, P<0.05.Whereas the rates in sandy soil and mixed soil were 8.91 % and 4.08 % respectively. Correlation between helminthes distribution, types of collected soil samples and type of employed laboratory techniques was significant.

To demonstrate role of soil samples in parasitic distribution, soil samples were examined using two described methods; It is

obvious from table 6 below that soil samples from urban area were highly contaminated with protozoan parasites specially the cysts of *Giardia lamblia* and *Cryptosporidium parvum* oocysts with equal rate 11.02 % for each, followed by 8.97 % and 7.34 % for trophozoites of *Trichomonas hominis* and oocycsts of *Cyclospora cayetanensis* respectively compare to lowest rates with other protozoa recorded in current study, P<0.05.Also the same finding was obtained with protozoa isolated from rural area. Relationship between protozoan distribution and site of soil sampling was significant (P<0.05).

Table.6 Protozoan parasites distribution according to site of sampling

Types of protozoan parasites	Rural location		Urban location	
	No.	%	No.	%
Cyclospora cayitenisis	10	4.08	18	7.34
Cryptosporidium parvum	8	3.22	27	11.02*
Giardia lamblia	20	8.16	27	11.02*
Blastocyst hominis	0	0.00	1	0.40
Trichomonas hominis	10	4.08	22	8.97
Balantidium coli	2	0.82	0	0.00
Toxocara canis	0	0.0	1	0.40
Microspora spp	1	0.40	3	1.22
Isospora spp	0	0.00	1	0.40
Giardia canis	1	0.40	0	0.00
Total of protozoa	52	21.22	100	40.81**

Total soil samples examined=245 *,** P<0.05.

Table.7 Frequency of parasitic helminthes according to site of soil samples

Helminthes	Rural area positive samples		Urban area positive samples **	
	No	%	No	%
Ascaris lumbricoides	6	2.44	10	4.08
Ancylostoma sp	6	2.44	10	4.08
Strongyloides stercoralis	15	6.12	27	11.02*
Lumbricus terrestris	0	0.00	3	1.22
Taenia spp	0	0.00	2	0.81
Enterobius vermicularis	1	0.40	3	1.22
Osterusovis	0	0.00	5	2.04
Physalopetra preaputialis	0	0.00	1	0.40
Echinocaccus spp	0	0.00	1	0.40
Total of helminthes	28	11.42	62	25.31

Total soil samples examined=245 *,** P<0.05.

Regarding occurrence of parasitic helminthes in urban and rural areas, the rate high 25.31 % of helminthes was recorded in urban area compare to 11.42 % from rural area, P <0.05.The common helminthes with high dominancy in compare to others involve *Strongyloides stercoralis 11.02%*, *Ascaris lumbricoides* 4.08 % and *Ancylostoma* species 4.08 %. Also the same three species were found in high rates in rural area, but there rates were half of those in urban areas. Rare records involve *Osterusovis* larvae 5/2.04 % and *Physaloptera preaputialis* 1/0.40 %. Relationship between parasite residencies and type of soil samples was significant (P<0.05).

The exposure of Iraq to different consequence wars, economic sanction after the war of 1991, changing style of government and policy after 2003 altered social net of Iraqi people particularly Kirkuk Province. These factors influence in high grade Kirkuk province, because geographically this Province located at the end of northern of Iraq that is communication to other close cities such as Baghdad, Dyiala, Tikrit. In order to avoid the war most of peoples in these Provinces were left their places and migrate to Kirkuk city and becomes displaced people distributed in Kirkuk randomly. These factors can explain why this city crowded and the rate of infectious agents becomes high particularly intestinal parasites, toxoplasmosis and leishmaniasis (Mustafa, 2013; Salman and Mohammad, 2015; Karyaghdi, 2013). The overall rate of parasites found in current study was not agreed with those reported in the same province by Karyaghdi (2013), Salman (2014), Salman and Salih (2013), Salman *et al.* (2001), Kadir and Salman (1998). Whom they record the following rates 62.16%, 52.07%, 65.59%, 36.7% and 51.2 %. Variances in the rates might be factorial such most of these studied were not pure of soil parasites (stool +soil), size of study, type of laboratory methods employee and wars effects during the processing current study.

Considering 62.03 % of protozoan parasites recording higher than 36.74 % for helminthes this difference between mentioned rates mostly due to breakdown of municipal water pipes underground during new roads construction in all parts of Kirkuk city. Actually this step had role in distribution of infectious agents within soil particularly *Giardia lamblia* 20 %, *Cryptosporidium* 14.28 % and 11.02 % of *Cyclospora* oocysts. While helminthes rate lower than protozoan rate might be due to climate changes in Iraq particularly during 2014-2015 especially in hot summer within which temperatures reach more than 45C.Normally this elevated temperature will kill the larvae of helminthes (nematodes) in superficial surface of soil (Gugino *et al.*, 2009). This elevated prevalence of protozoan parasites in soil samples of Kirkuk Province was reflects the poor hygienic condition, low level of sanitation particularly in area were displaced peoples were live and prognoses to future outbreaks of giardiasis, cryptosporidosis and cyclosporoisisin case when, there is no improvement of health and environment in Kirkuk province. The elevated rates of Giardia and cryptosporidium in current study was higher than those recorded in the same Province by Mustafa,2003and Al-Bayati,2011, whom they20.62% and 8.89 % of *Giardia lamblia* (Mustafa, 2013; Al-Baiti, 2011). Also not agreed with Othman (2000), who record 21.24 % of *Cryptosporidium* oocysts in stool samples among children in Kirkuk city.

Considering *Cyclospora*rate 11.02% in current study was very high which is high lightning the shadow on diarrhea causes in

this province, because most of diarrheic stool samples were negative for intestinal parasites, bacterial, viral infections. For this reason *Cyclospora*as one cause of diarrhea in recent time should be taken in consider and training programs for laboratory technician s on the diagnosis and recognition of this parasite essential to be carrying on.

Soil samples were not searched *for Cyclospora* previously, so its rate comparison can be done with other research close to soil because soil is a medium in which most of parasites were propagated including *Cyclospora* (Chacín-Bonilla, 2010). *Cyclospora* rate in current study was higher than those recorded by (Mansfield and Gajadhar, 2004; Al-Aakely, 2007; Al-Samarrai, 2008; Nimri, 2003; El-Karamay *et al.,* 2005). This finding not reveal only the role of soil in parasite transmission, but other vehicles of transmission such as food and water contamination with the infective stage "the oocyst" had role in this elevated rate (Helmi, 2010).

On the other hand, protozoan parasites recorded rate 39.58 % by using double wet preparations than 27.75% using the flotation technique regarding *Cyclospora*is critical for parasite diagnosis as it was found that concentration methods including flotation technique are confirmatory to direct examination of stool samples by wet preparations. The explanation to that is might be due technical errors such as an excess washing, centrifugation of samples, preservation, $ZnSO_4$ solution preparation, sample arrival to lab (WHO, 1991).

Moreover low rates of *Toxocara* species ova, *Isospora*, *Balantidium coli* in current study mostly due strong role of veterinarian Hospital activity in killing stray dogs and cats in this province, while *Balantidium coli* low rate can be attributed to absence of swine inside Kirkuk city and two cases positive were recorded in rural area. Infections with soil-transmitted helminthes (STH) *Ascaris lumbricoides, Trichuris trichiura*, and the hookworms (*Ancylostoma duodenale* and *Necator americanus*) are acknowledged as neglected tropical diseases. They result in a huge public health burden, affecting more than one billion people worldwide (de Silva *et al.,* 2003). Infections with *Strongyloides stercoralis*, another STH, which affects 30 to 100 million people worldwide, are probably even more underestimated (Bethony *et al.,* 2006). The effects of intestinal helminthes infections depend on several factors such as the helminthes species, intensity of the infection, and the host immunological status. These infections can result in chronic effects on health and nutritional status of the host, especially among children and immunocompromised individuals. Anemia, malnutrition, and gastrointestinal or pulmonary complaints are some of the problems associated with intestinal helminthes infections (Hotez *et al.,* 2004). In addition, fatal cases are seen with autoinfection by *S. stercoralis* because it may result in hyper-infection (Marcos *et al.,* 2008). For these reasons recording high rate of *Strongyloides, Ascaris* and *Ancylostoma* in current study was vital and more benefit to whom they are interest in community medicine in order to carry on further studies on soil and soil parasites to reduce rate of STH.

Variances in the rates recovering the parasites according to three types of soil samples in current study statistically important because both protozoan 35.91 % and helminthes 22.85 % when they compare to mixed soil samples 15.10% and 4.08% for both protozoan and helminthic parasites respectively. The cause to that might be related to high humidity in clay soil than mixed one, which can encourage the oocyst shedding and preservation. Also clay type

soil nature is smooth that permit parasite movement than mixed or sandy soil which have had rough granules may prevent or block parasites movements (Chacín-Bonilla, 2008; Lopez *et al.,* 2003). Also the importance of clay type soil was clear in recording some rare helminthes such as *Physoleptra preputalis*, *osterusovis* and *Echinococcus*ova, by them the chance of getting cutaneous creeping eruption (Singh and Rao, 1954), Myiasis, skin deformities and hydatid cyst were possible respectively in human when comes contact with contaminated clay soil.

Exerting high rates of protozoan and helminthic parasites in soil samples from urban area than in rural area in current study was critical, it reflects the degree of environment contamination in Kirkuk city with parasitic forms than rural area. The initial source of contamination of the urban area in Kirkuk Province is still unknown. Possibilities include the soil, use of contaminated water for irrigation and pesticide dilution, and the poor sanitary facilities available to seasonal field workers (Shield and Olson, 2003). Furthermore it can be explained by an excess crowding due to attending of displaced people from neighboring Provinces due to war. In addition to continues electric and water interruption in Kirkuk city which can accelerate stored food disturbance in houses, vegetables washing and even personal cleaning and water consumption (Döller *et al.,* 2002).

Conclusions: Soil samples in Kirkuk Province were highly contaminated with parasites particularly clay soil type. *Cyclospora cayetanensis* oocysts act as one causes of soil contamination in Kirkuk province. Direct double wet preparations of soil samples were with high efficacy in demonstrating protozoan parasites particularly *Cyclospora cayetanensis* than flotation technique.

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