Isolation and Diagnosis of Parasites from Different Soils in Baghdad City

Maisam Balasim Al-Khamesi
College of Science for Women, Baghdad University.

Abstract

A study was conducted to determine the prevalence of parasites in soil texture (loam) by using four techniques (salt floatation, zinc sulphate floatation, sedimentation and filter paper technique). During the period from December 2012 to May 2013, 70 soil samples were collected and analyzed from six various sites of Baghdad City such as (house garden, vicinity of house, vicinity of gutter, waste dumps, vegetable farm and hospital garden like Alyarmouk teaching hospital and Central teaching hospital of pediatric). The prevalence of soil parasites was as follows: *Toxocara* spp. eggs isolated by two floatation techniques (salt and zinc floatation techniques), but this parasite appeared significant in its floatation by salt floatation technique (p= 0.042), while protozoan cysts appeared highly significant in zinc floatation technique (p= 0.00001). In addition, larvae of *Strongloides stercoralis* showed highly significance in sedimentation and filter paper technique (p=0.0001 and p= 0.001) respectively. Furtherly, female of *Strongloides stercoralis* appeared significant in its isolation in sedimentation (p= 0.0463), *Ascaris lumbricoides* (ova) showed highly significant in sedimentation technique (p= 0.0043). These results concluded that the *Toxocara* spp. eggs more prevelant than other parasites in soil samples, and the soil may play an important role in transmission of zoonotic parasite diseases to human. In addition, the control of high population of animals such as stray dogs and cats is necessary to reduce the distribution of parasites.

Keywords: *Toxocara*, *Strongloides*, *Ascaris*, floatation techniques.

Introduction

Soil transmitte parasites are the largest group of parasites that live in the soil during their development [1]. Contamination of soil with parasite eggs, infective larvae, cystsand oocysts constitutes the most important risk factor for zoonotic parasite infection [2]. Zoonotic parasites (that is, *Toxocara spp*.) and geohelminths (that is, *Ascaris lumbricoides*, *Trichuris trichura* and hookworms) are animal parasites that can infect humans especially through contact with contaminated soil [3,4]. Parasitism is the major health problem both for animals and humans, which constitutes major part of the zoonoses. most zoonotic parasites are found worldwide, they are more prevalent in tropical and subtropical regions where populations experience poor socioeconomic conditions [5,6]. soil transmitted helminth infections are common chronic human infections world-wide [7]. The major obstacle to the implementation of cost-effective control is the lack of accurate descriptions of the geographical distribution of infection [8,9].

The objective of this study is to determine the prevalence of parasitic forms (eggs, larvae, cysts) in soil loam of Baghdad City.

Materials and Methods

Samples

During the period froms December 2012 to May 2013, 70 soil samples (soil loam) were collected from various sites in Baghdad city. The sites included house garden, vicinity of house, vicinity of gutter, waste dumps, vegetable farms and hospitals.

Procedures

1. Saturated salt floatation technique

A simple and popular method is salt floation using a saturated solution of sodium chloride, having a specific gravity of 1.2. About 2 ml of the salt solution is taken in a flat bottomed tube and 1gram of soil is emulsified in it. The container is then filled completely to the brim with the salt solution and a slide is placed on the container so that it can be in contact with the surface of the solution, without any intervening air bubbles after standing for 20-30 minutes, the slide is remove, without jerking, reversed to bring the wet surface on top and examined under the microscope after mixing with a drop of iodine. [10].

2. Zinc sulphate centrifugal technique

About 1gram of soil was thorouly mixed in 10 ml distilled water. The coars particles are removed by straining through gausze. The filtrate is poured into 15ml conical centrifuge tube and centrifuged at 2500 (r.p.m.) for 1 minute. The supernatant fluid is poured off and distilled water is added to the sediment. It is shaken well, centrifuged and the process is repeated 2 or 3 times till the supernatant fluid is clear. The clear supernatant is poured off and 3-4 ml of zinc sulphate (specific gravity 1.18) is added to the sediment and more zinc sulphate solution is added to fill the tube up to the top and centrifuged again at 2500 (r p m) for 1 minute. With a platinum wire loop sample is taken from the surface, on to a clean glass slide, a coverslip is put on and examined under the microscope after mixing with a drop of iodine [11].

3. Sedimentation Methods (Formalin-ether sedimentation)

Formol – ether concentration method has been the most widely used sedimentation method. Emulsify 1-2 gram soil in 10 ml distilled water and strained through two layers of gauze in a funnel. The filtrate is centrifuged at 2500 (r p m) for 2 minutes. The supernatant is discarded and the sediment is resuspended in 7 ml of formol saline(10%), allowed to stand for 10 minutes, then added 3 ml of ether, the tube is stoppered and shaken vigorously to mix. Then the stopper is removed and centrifuged at 2500 (r p m) for 2 minutes. The tube was allowed to rest in a stand. Four layers became visible, the top layer consisted of ether, the second was a plug of debris, the third was a clear layer of formol saline and the fourth is sediment. The plug of debris was detached from the side of the tube with the aid of a glass rod and the liquid was poured off leaving a small amount of formol saline for suspension of the sediment. It was poured on a clean glass slide, covered with coverslip and examined under the microscope after mixing with a drop of iodine [10,11].

4. Filter paper / slant culture technique

In the center of each filter paper strip, smear relatively thin film of 1 to 2 gram of soil, then this strip was placed on the slide, which is inclined at one end in the petri dish by a piece of glass tubing. After that, a shallow layer of water is added to the dish so that the bottom portion of the slide immersed in water. The soil will be kept moist by capillary action and the dish will keep covered and at room temperature. The culture may be examined directly under the microscope in addition to examine the water surrounding the slide [12].

Statistical Analysis

Statistical analysis was comute assisted using SAS (Statistical Analysis System). In addition to using (LSD) for variables by P-values [13].

Results and Discussion

This work was aimed to determine the prevalence of all parasitic forms by using (salt floatation, zinc sulphate centrifugal floatation, sedimentation and filter paper technique) in soil samples.

Table (1) and Fig.(1 A) showed that *Toxocara* spp. eggs which were highly significant distribution in all locations. On the other hand, this parasite was isolated by two floatation techniques (salt and zinc sulphate floatation), because the suspension in a solution of high spesific gravity so that parasitic eggs float up and get concentrated at the surface [10], and it is isolated significantly by salt floatation technique (p=0.042).

Furtherly, this table showed *Toxocara* spp. eggs was more prevalent than other parasites in soil samples of Baghdad City, the higher incidence recorded in the present study may be due to the factors like geographic and climatic conditions of country, poor management practices and frequent mixing of pets with stray dogs which might have the infections, this result was similar to [14].

Table (1)
Effect of techniques and location of samples collection on the presence of Toxocariasis spp. (ova).

Location of samples collection	Techniques				P-value
	Saturated salt floatation	Zinc sulphate centrifugal floatation	Sedimentation	Filter paper	
House garden	++++	+++	0	0	0.0034**
Vicinity of house	+++++	++++	0	0	0.0001**
Vicinity of gutter	+++++	+++	0	0	0.0001**
Waste dumps	++++	+++	0	0	0.0034**
Vegetable farms	++++++	+++	0	0	0.0001**
hospital garden	++++	++++	0	0	0.0019**
P-value	0.042*	NS	NS	NS	---
(P≤0.01)** ،(P≤0.05) *, NS: Non Significant ++++ : 4 posative samples, +++++ : 5 posative samples,…….etc.					

As shown in Table (2) and Fig.(2 A) the prevalence of protozoan cysts were highly significant in vicinity of gutter (p=0.0037), waste dumps (p=0.0001) and vegetable farms (p=0.00001) compared with other locations. In addition, this parasite showed highly significant isolation by zinc sulphate floatation technique (p=0.00001), due to the specimen in a medium of greater density than that of protozoan cyst [11]. This result was supported by [15].

Table (2)
Effect of techniques and location of samples collection on the presence of Protozoan (cyst).

Location of samples collection	Techniques				P-value
	Saturated salt floatation	Zinc sulphate centrifugal floatation	Sedimentation	Filter paper	
House garden	0	+	0	0	NS
Vicinity of house	0	+	0	0	NS
Vicinity of gutter	0	++++	0	0	0.0037**
Waste dumps	0	+++++	0	0	0.0001**
Vegetable farms	0	++++++++	0	0	0.00001**
Hospital garden	0	0	0	0	NS
P-value	NS	0.00001**	NS	NS	---
(P≤0.01)** , NS: Non Significant + : 1 posative sample, ++++ : 4 posative sample, +++++ : 5 posative sample, …….etc.					

Table (3) and Fig.(1 B), showed that the distribution of larvae of *Strongloides stercoralis* showed, highly significance in vicinity of gutter (p=0.0001), while appeared significantly in its distribution in house garden (p=o.o463), vicinity of house (p=0.0463) and vegetable farms (p=0.0274), due to the soils of these gardens espicially in vegetable farms also harbour helminthic parasites as it is contaminated with human excreta, especially in places where it is used as manur. This result was similar to [16, 17]. On the other hand, the isolation of this parasite showed highly significant in sedimentation technique (p=0.0001) and filter paper technique (p=0.001) compared with other techniques because it is suspended in a solution with low –specific gravity so that the larvae can get sedimented at the bottom [10]. This result is similar to [18].

Table (3)
Effect of techniques and location of samples collection on the presence of Strongloides stercoralis larvae.

Location of samples collection	Techniques				P-value
	Saturated salt floatation	Zinc sulphate centrifugal floatation	Sedimentation	Filter paper	
House garden	0	0	++	0	0.0463*
Vicinity of house	0	0	+	++	0.0463*
Vicinity of gutter	0	0	++++++	++++	0.0001**
Waste dumps	0	0	0	0	NS
Vegetable farms	0	0	0	+++	0.0274*
Hospital garden	0	0	+	0	NS
P-value	NS	NS	0.0001**	0.001**	---
(P≤0.01)** ،(P≤0.05) * , NS: Non Significant + : 1 posative sample, ++ : 2 posative samples, ++++++ : 6 posative samples,……etc.					

Table (4) and Fig.(1 C) showed the isolation of *Strongloides stercoralis* female which increased significantly in house garden (p=0.0463) and waste dumps (p=0.0463) due to this parasite that is a geohelminth having free–living life cycle in warm humid soil [19]; in addition, this parasite is isolated by two techniques (sedimentation and filter paper technique), but it is showed significance in its isolation by sedimentation technique (0.0463) compared with other techniques because this parasite has greater density than the suspended medium [11]. This result was similar to [20].

Table (4)
Effect of techniques and location of samples collection on the presence of Strongloides stercoralis female.

Location of samples collection	Techniques				P-value
	Saturated salt floatation	Zinc sulphate centrifugal floatation	Sedimentation	Filter paper	
House garden	0	0	++	0	0.0463*
Vicinity of house	0	0	+	+	NS
Vicinity of gutter	0	0	+	0	NS
Waste dumps	0	0	++	+	0.0463*
Vegetable farms	0	0	+	+	NS
Hospital garden	0	0	0	0	NS
P-value	NS	NS	0.0463*	NS	---
(P≤0.05) * , NS: Non Significant + : 1 posative sample, ++ : 2 posative samples					

The presence of *Ascaris lumbricoides* (ova) which appeared in Table (5) and Fig.(2 B), showed highly significance in house garden (p=0.0037). This may be due to the using of feces as fertilizer which was avoided and could be a factor of low frequency of finding this parasite [21]. While this table showed increased significantly in vicinity of house (p=0.0274) and waste dumps (p=0.0463). On the other hand, the isolation of *Ascaris lumbricoides* (ova) showed highly significance by sedimentation technique (p=0.0043) because the suspension in a solution with low – specific gravity so that the eggs can get sedimented at the bottom [10]. This result is supported by [18].

Table (5)
Effect of techniques and location of samples collection on presence of Ascaris lumbricoides (ova).

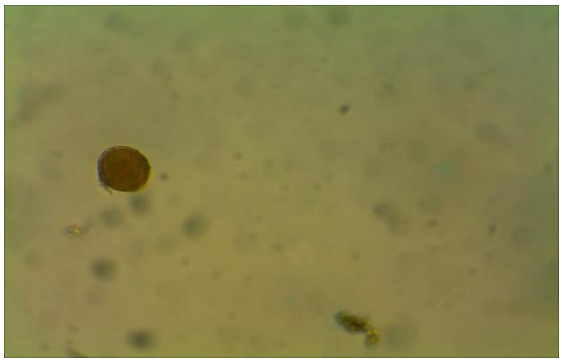
Location of samples collection	*Techniques*				*P-value*
	Saturated salt floatation	*Zinc sulphate centrifugal floatation*	*Sedimentation*	*Filter paper*	
House garden	0	0	++++	0	0.0037**
Vicinity of house	0	0	+++	0	0.0274*
Vicinity of gutter	0	0	+	0	NS
Waste dumps	0	0	++	0	0.0463*
Vegetable farms	0	0	+	0	NS
Hospital garden	0	0	0	0	NS
P-value	NS	NS	0.0043**	NS	---
(P≤0.01)** ،(P≤0.05) * , NS: Non Significant + : 1 posative sample, ++ : 2 posative samples, +++ : 3 posative samples,…….etc.					

A

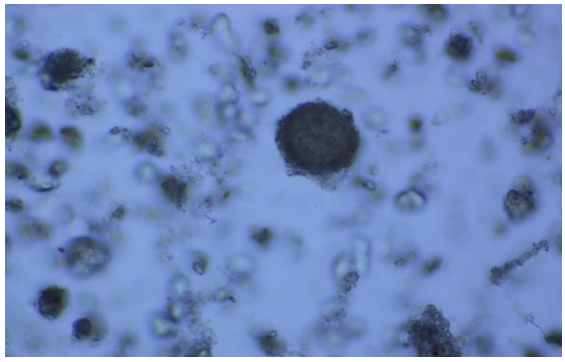
B

C

Fig.(1) A-Ova of Toxocariasis, B- Larvae of Strongloides stercoralis, C- Female of Strongloides stercoralis.



A



B

Fig.(2) A- Protozoan cyst, B – Ova of Ascaris lumbricoides.

In conclusion, the results of this study on soil contamination by parasites may play an important role in transmission of these zoonotic parasitc diseases to human. The control of population of animals such as stray dogs and cats is necessary to reduce the distribution of parasites.

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الخلاصة

اجريت الدراسة لغرض التحري عن انتشار الطفيليات في التربة المزيجية وذلك بأستخدام اربع تقنيات للعزل (تقنية التطويف بالمحلول الملحي و التطويف بسلفات الزنك و الترسيب و استخدام اوراق الترشيح) .تم جمع ودراسة ٧٠ عينة تربة للفترة من كانون الأول ٢٠١٢ ولغاية ايار ٢٠١٣ من ستة اماكن مختلفة من مدينة بغداد (حديقة منزل وبالقرب من المنزل وبالقرب من مياه اسنة وبالقرب من نفايات وحقل خضراوات ومن حديقة المستشفى مثل مستشفى اليرموك التعليمي ومستشفى الطفل المركزي). عزلت بيوض طفيلي *Toxocara* spp. بطريقتي التطويف (المحلول الملحي وسلفات الزنك) ولكنها اظهرت نتائج معنوية بطريقة التطويف بالمحلول الملحي (p=0.042)، بينما عزلت اكياس الأبتدائيات بمعنوية عالية بطريقة التطويف بسلفات الزنك (p=0.00001)، اضافة الى ان يرقات طفيلي *Strongloides stercoralis* اظهرت معنوية عالية بطريقتي الترسيب واستخدام اوراق الترشيح (p=0.0001, p=0.001) بالتعاقب. كما عزلت اناث طفيلي *Strongloides stercoralis* بطريقتي الترسيب واستخدام اوراق الترشيح ولكنها بينت نتائج معنوية بطريقة الترسيب (p=0.0463)، واخيرا عزلت بيوض طفيلي *Ascaris lumbricoides* وبمعنوية عالية بطريقة الترسيب (p=0.0043)، من جهة اخرى بينت النتائج ان طفيلي الـ *Toxocara* spp. هو الأكثر انتشارا في التربة من بقية الطفيليات. يمكن الأستنتاج ان التربة ممكن ان تلعب دورا هاما في نقل الأمراض الطفيلية للأنسان، لذا فأن السيطرة على الحيوانات مثل الكلاب والقطط السائبة مهم جدا للحد من انتشار هذه الطفيليات.