Prevalence of Parasites in Soil by two Floatation Techniques and Modified Acid-Fast stain in Baghdad city

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Abstract

The objective of the present study is to determine the prevalence of parasitic forms such as (eggs, Oocysts,….etc) in soil texture (loam) during the period from September 2013 to March 2014 by using two floatation techniques (Saturated salt floatation and Sheather´s floatation techniques), in addition to the Modified acid fast stain technique to detect Oocyst. 100 soil samples were collected from different locations and random areas in (50 soil samples from Al-Karch & 50 soil samples from Al-Rusafa) for isolation the parasites by two floatation techniques, and modified acid fast stain method in Baghdad city. Our result showed that, *Toxocara* spp. eggs isolated by two floatation techniques in (48.00%&42.00%) from Al-Karch & Al-Rusafa places respectively, in addition to the Chi-Square appeared highly significant for this isolation (9.25)($p\leq0.01$). While, the isolation of other parasites such as (Ova of cestodes and male&female of nematodes) occured by Sheather´s floatation technique only so, these results showed the ova of cestodes appeared (18.00%&16.00%) in two areas, furtherly, the Chi-Square showed highly significant (14.50)($p\leq0.01$) in isolation. While male & female of nematodes appeared (16.00%&10.00%) in two areas, in addition to the Chi-Square appeared highly significant (14.50)($p\leq0.01$). On the other hand from this study, the isolation of Oocyst from (soil texture samples also taken from different locations and random areas in Al-Karch & Al-Rusafa), the result showed (16.00%&18.00%) in two areas respectively, and appeared highly significant (12.96%&12.51) in Chi-

Square for this parasite .We conclude from this study that the soil is the main reservoir of different parasites, and humans in both areas are at risk of acquiring helminth infection from contaminated soil.

Key words-: Soil contamination, Practical Parasitology, Nematodes in soil, Prevalence of parasites.

Introduction

Parasitic helminthes are known to be endemic in developing countries, owing to poor environmental sanitation in communities, improper disposal of waste (including human feces and other organic waste)[1,2]. Studies from various countries have demonstrated a high rate of soil and grass contamination with infective parasitic elements in public and urban areas[3,4]. Intestinal parasites including soil-transmitted helminthes (STH) have been long recognized as public health problems in much of the developing world[5]. Soil-transmitted helminthiases are caused by four species of intestinal worms, namely, *Ascaris lumbricoides*, *Trichuris trichiura*, and two hookworm species (*Ancylostoma duodenale* and *Necator americanus*)[6]. Current estimates show that more than 2 billion people worldwide are infected with STH, the majority residing in low and middle income countries[7]. Intestinal canine and feline parasitic infections transmissible to humans, such as toxocariosis, giardiosis,…etc, have a cosmopolitan distribution and are among the ten most common infections in the world[8,9]. Mainly in developing countries, so called soil-transmitted helminthes still remain a formidable public health problem of cosmopolitan importance[10].

The aim of this study is to investigate the prevalence of parasites in soil texture by two floatation techniques (Saturated salt floatation & Sheather´s floatation) and Modified Acid-Fast stain technique in Baghdad city.

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Materials & Methods

- Samples Collection:-

A total of 100 soil samples (soil texture) were collected from various places in Al-Karch such as(Hay-Al- Jameaa, Al- Doura, Al- Saydiyia, Abu-disheer, Al-Kadraa, Hay- Al-Adel, Al-Usifyia & Al-Shuala) and Al-Rusafa such as(Al-Karada, Al-Jadiryia, Al-Salam, Madinat Al-Sader, Baghdad Al-Jadeeda, Al-Baladiyat, Al-Turath & Zaiuona) of Baghdad city, during the period from September 2013 to March 2014.

-Procedures:-

١ . Sheather´s floatation technique

* Sheather´s solution:- Add 4gm of table sugar to 355ml of hot water and then add 6ml of formalin to prevent mold growth. Stir until dissolved and allowed to cool then kept refrigerated.

Method:-

Add 10ml of Sheather´s solution in test tube, then weight 3gm of soil and emulsified in Sheather´s solution. The coarse particles are removed by strainer through layers of gauze in a funnel. The filtrate was centrifuged at (2500 r.p.m.) for 2-4 minutes, then the test tube filled completely to the brim with Sheather´s solution and placed a slide on the test tube, after that let sit for about 5 minutes, then remove it and mix it with a drop of iodine and examin it under the microscope[11].

2. Saturated salt floatation technique

A simple and popular method is salt flotation 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 removed, without jerking, reversed to bring the wet surface on top and examined under the microscope after mixing with a drop of iodine.[12].

3. Modified Acid – Fast Stain technique

A thin smear of soil was made on a clean glass slide. It was fixed by heat at 70^{o}C for 10 minutes. It was kept on the staining rack and flooded with carbol fuchsin. The slide was heated till carbol fuchsin starts steaming. More carbol fuchsin was added to prevent slide from drying. The slide was allowed to stain for 9 minutes and washed with tap water. It was then decolourised with 5% aqueous sulphuric acid for 30 seconds, followed by washing with tap water and counter staining it with methylene blue for 1 minute. Finally it was washed with tap water, dried and examined under the microscope[13].

Statistical Analysis

Statistical analysis was comuted using SAS (Statistical Analysis System) .In addition to using (Chi-Square) for comparing the statistical significant between percentages [14].

Results & Discussion

Our survey concentrated on parasitic elements which represented a risk for human health by using two floatation methods (saturated salt & Sheather´s floatation technique), in addition to Modified Acid-Fast stain

technique to recovery of Oocysts. Table-1- Figure1,A showed the isolation of Ova of *Toxocara* spp. by two floatation techniques from 100 soil samples (50 from Al-Karch & 50 from Al-Rusafa places) which were mentioned above, this method appeared (48.00% & 42.00%) as positive results in addition to (52.00% & 58.00%) as negative results in Al-Karch & Al-Rusafa places respectively, in the same time the Chi-Square appeared highly significant (9.25)($p\leq0.01$) in two places for isolation this parasite, while when we compared between them by Chi-Square showed non significant. Furtherly, this table showed the isolation of Ova of *Toxocara* spp. by saturated salt floatation technique was more than Sheather´s floatation technique in two places. This parasites isolation by floatation techniques, because the soil material was dissolved in solution of a higher density than that of the parasite [15]. Also this study showed the Ova of *Toxocara* spp. was the most frequent finding and had the highest prevalence in two places may be depending on climate and geographic differences, in addition, the level of contamination varied in different places and within it were determined by local factors, this result confirmed by [16].

Table-1- Numbers & percentages of *Toxocara* spp. in saturated salt & Sheather´s floatation techniques in Al-Karch & Al-Rusafa areas:-

The area	Salt floatation technique		Sheather´s floatation techniques		Positive	Negative	Chi-square
	No.	%	No.	%			
Al-Karch (50)	16	66.67	8	33.33	24 (48:00%)	26 (52.00%)	9.25**
Al-Rusafa (50)	14	66.67	7	33.33	21 (42.00%)	29 (58.00%)	9.25**
Chi-square	----	NS	----	NS	NS	NS	-----
($P\leq0.01$)**, NS: Non Significant							

As shown in table-2- figure-1,B the isolation of cestodes (ova) appeared (100.00%) by Sheather´s floatation technique so, the positive result for this parasite showed (18.00% & 16.00%) and (82.00% & 84.00%) as a negative result in Al-Karch & Al-Rusafa respectively. Furtherly, the Chi-Square appeared highly significant (14.50) ($p\leq0.01$) in two places for isolation of this parasite, on the other hand the comparison between them by Chi-Square showed non significant for this parasite. The isolation for this

parasite by Sheather´s floatation technique may be due to their relatively small size, high specific gravity of solution and subsequent tendency to settle at the same level as various pigments and debris, this result agrees with[17].

Table-2- Numbers & percentages of Cestodes (Ova) in saturated salt & Sheather´s floatation techniques in Al-Karch & Al-Rusafa areas:-

The area	Salt floatation technique		Sheather´s floatation techniques		Positive	Negative	Chi-square
	No.	%	No.	%			
Al-Karch (50)	٠	0.00	٩	100.00	9 (18.00%)	41 (82.00%)	14.50**
Al-Rusafa (50)	٠	0.00	٨	100.00	8 (16.00%)	42 (84.00%)	14.50**
Chi-square	---	NS	---	NS	NS	NS	-----
(P≤0.01)**, NS: Non Significant							

Table-3- figure-1,C&D also showed the isolation of nematodes(male & female) occurred by Sheather´s floatation technique in (100.00%). So, the positive result for isolation of this parasite appeared (16.00% & 10.00%) in addition to the negative result showed (84.00% & 90.00%) in Al-Karch & Al-Rusafa respectively, in the same time the Chi-Square showed highly significant (14.50)(p≤0.01), while when we compared between them by Chi-Square, appeared non significant for isolation of this parasite. The prevalence and isolation of nematodes from soil (may be due to the soil, an excellent habitat for nematodes because of their importance to agriculture) by Sheather´s floatation which was best used with soil samples, this result is similar to[18].

Table-3- Numbers & percentages of Nematodes (male&female) in saturated salt & Sheather´s floatation techniques in Al-Karch & Al-Rusafa areas:-

The area	Salt floatation technique		Sheather´s floatation techniques		Positive	Negative	Chi-square
	No.	%	No.	%			
Al-Karch (50)	٠	0.00	8	100.00	8 (16.00%)	42 (84.00%)	14.50**
Al-Rusafa (50)	٠	0.00	٥	100.00	5 (10.00%)	45 (90.00%)	14.50**
Chi-square	---	NS	---	NS	NS	NS	----
(P≤0.01)**, NS: Non Significant							

On the other hand of this study, Table-4- figure-1,E&F showed the isolation of Oocyst from 100 soil texture samples collected from different locations in Al-Karch & Al-Rusafa which were mentioned above by using Modified Acid – Fast stain, appeared (16.00% & 18.00%)) as positive results, while showed (84.00% & 82.00%) as a negative result in two places, so the Chi-Square appeared highly significant (12.96 & 12.51))($p \leq 0.01$) in isolati[B]f Oocyst from soil sample[C]urtherly, the comparison between collected places (Al-Karch & A[A]safa) showed failure to reach the level of statistical significant. The present of Oocyst in soil samples may be due to the sporulated Oocyst may survive for long periods, depending on environmental factors, and resistant to some disinfectants commonly used around livestock, this result agrees with[19].

Table-4- Numbers & percentages of Oocyst in Modified Acid Fast Stain method in Al-Karch & Al-Rusafa areas:-

The area	Positive test		Negative test		Chi-square
	No.	%	No.	%	
Al-Karch (50)	8	16.00	٤٢	84.00	12.96**
Al-Rusafa (50)	٩	18.00	٤١	82.00	12.51**
Chi-square	-----	NS	----	NS	-----
(P≤0.01)**, NS: Non Significant					

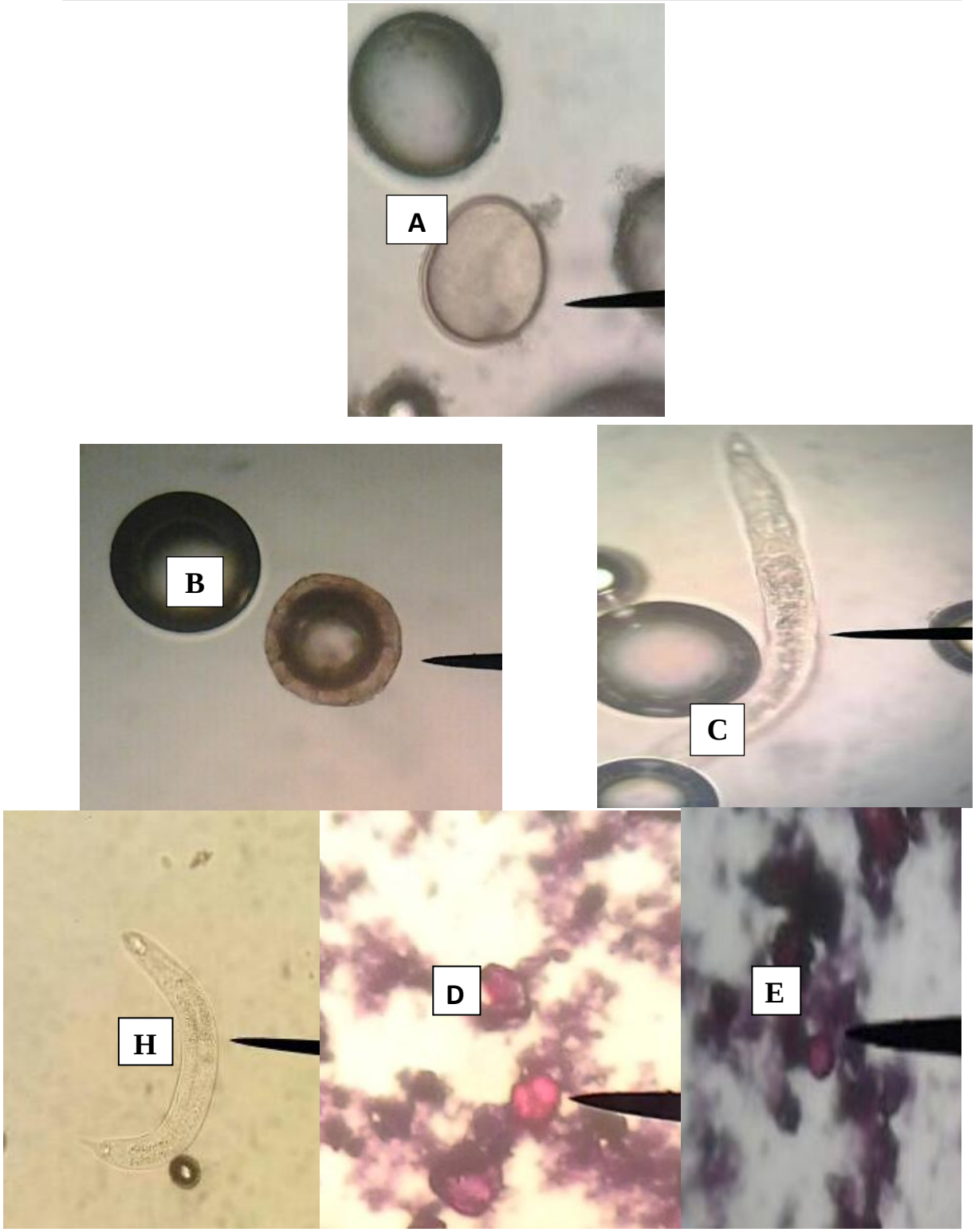

Figure-1- A – *Toxocara* spp. (ova), B - Ova of Cestodes, C- Female of Nematodes, D- Male of Nematodes, E & F- Oocyst in soil smear.

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انتشار الطفيليات في التربة بأستعمال طريقتين للتطويف فضلا عن الصبغة المقاومة للحامض

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الخلاصة

اجريت الدراسة بهدف التحري عن انتشار الأشكال الطفيلية مثل (البيوض، وأكياس البيوض....الخ) في التربة المزيجية للفترة الممتدة من ايلول ٢٠١٣ ولغاية آذار ٢٠١٤ وذلك بأستعمال طريقتين للتطويف (المحلول الملحي المشبع والمحلول السكري) فضلا عن استعمال طريقة الصبغة المقاومة للحامض للتحري عن وجود Oocysts.

تم جمع ودراسة ١٠٠ عينة تربة مزيجية من أماكن مختلفة وعشوائية لمناطق الكرخ (٥٠ عينة) ومناطق الرصافة (٥٠ عينة) لدراسة عزل الطفيليات بأستعمال محلولي التطويف، فضلا عن استعمال طريقة الصبغة المقاومة للحامض من مدينة بغداد.

أوضحت النتائج ان بيوض طفيلي .*Toxocara* spp عزلت بأستعمال طريقتي التطويف (الملحي والسكري) بنسب (٤٨,٠٠% & ٤٢,٠٠%) لكلا المنطقتين بالتعاقب، اضافة لذلك فأن قيمة مربع كاي اوضحت فروقا عالية المعنوية لعزل ذلك الطفيلي (٩,٢٥)($p\leq0.01$)، بينما عزلت باقي الطفيليات مثل (Ova of cestodes and Male&Female of Nematodes) بطريقة محلول التطويف السكري فقط، لذلك بينت النتائج بأن Ova of Cestodes عزلت بنسبة (١٨,٠٠% & ١٦,٠٠%) لكلا المنطقتين، اضافة الى ان قيمة مربع كاي بينت فروقا عالية المعنوية (١٤,٥٠)($p\leq0.01$) لعزل تلك الطفيليات. بينما Male&Female of Nematodes اوضحت الدراسة بأنها عزلت بنسب (١٦,٠٠% & ١٠,٠٠%) ولكلا المنطقتين كذلك فأن قيمة مربع كاي بينت فروقا عالية المعنوية للعزل (١٤,٥٠) ($p\leq0.01$) .

كما تم عزل Oocysts من مناطق مختلفة وعشوائية لترب مزيجية أيضا وقد بينت النتائج ان عزل الطفيلي المذكور أعلاه كان بنسب (١٦,٠٠% & ١٨,٠٠%) لكلا المنطقتين بالتعاقب

اضافة الى ان قيم مربع كاي اظهرت معنوية عالية (12.51 & 12.96)($p \leq 0.01$) ولكلا المنطقتين بالنسبة للطفيلي المعزول. لذا يستدل من هذه الدراسة بأن التربة هي الخازن الرئيسي لبيوض وأطوار الطفيليات المختلفة ويعتبر الإنسان في كلا المنطقتين معرض لخطر الإصابة بالديدان الطفيلية من الترب الملوثة بها.

الكلمات المفتاحية:– تلوث التربة، علم الطفيليات العملي، الديدان الخيطية في التربة، انتشار الطفيليات.