

A Coprological Diagnostic Comparison Between Zinc Sulphate Flootation and Formalin:Ether Sedimentation with Two Natural Extracts (Pomegranate Molasses and Honey)

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Abstract

A survey of the common human intestinal parasites was conducted during the period from October 2005 to March 2006. Out of 524 faecal samples were examined by concentration methods, which includes the centrifugal flotation by Zinc Sulphate solutions and centrifugal sedimentation by Formalin-Ether and also by using two natural extracts, one of them from the plant product (Pomegranate molasses) and the other from animal product (Honey), after examination of both extracts by concentration method revealed that the sediment have the ability to concentrate the cysts of protozoans and ova of helminths according to the specific gravity. Zinc Sulfate flotation method showed the higher efficiency in diagnosing intestinal parasites (36.64%), while among sedimentation methods which were used in the present study Pomegranate molasses showed the higher efficiency (30.77%) when compared to other methods also have ability for slightly staining the stages of the parasites. These methods of Pomegranate molasses and Honey sedimentation can be considered as the first attempt in this field.

Keywords: Flootation method, Zinc Sulphate, Sedimentation, Formalin:Ether, Natural-extracts, Intestinal parasites.

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Introduction

Parasitic infections are common medical problems in the tropics and sub-tropics, different laboratory methods were employed for their detection. However, comparative data are not available in sufficient quantity to determine the optimal technique [1].

In general, many studies have been done on intestinal parasites by using Zinc Sulphate flotation and Formaline:Ether sedimentation, and to the best of our knowledge no study has been done by using plant and animal

extract as a diagnostic methods for detecting intestinal parasites. [2] have examined 280 fecal specimens by Formalin:Ethyl ether sedimentation, Formalin:Ethyl acetate sedimentation, and Zinc Sulphate flotation techniques, 50 positive specimens were identified. Only slight differences in the detection of parasites were found for the three methods, and the results obtained with the Formalin:Ether and Formalin: Ethyl acetate procedures were identical. The recovery of *E.coli*, *G. lamblia* cysts and *H.*

nana ova was slightly better with the Zinc Sulphate procedure, but the detection of *Taenia* and *Ascaris* ova was somewhat better with the sedimentation procedures.

[3] have examined fecal samples (By direct and Formalin:Ether sedimentation methods) of schoolchildren aged 6-13 years old of both gender, the samples were taken from two different districts of Erbil-City (Koran and Azadi). Out of the total number of samples (424), the rate of infection was 62.74%, the rate of infection for Koran district (low socio-economic) was higher (77.585) than that of Azadi district (high socio-economic) 46.27%, they recorded *G. lamblia*, *E. histolytica*, *E. coli*, *H. nana* and *A. lumbricoides* in the rate 35.59%, 18.6%, 48.5%, 28.32% and 19.72% respectively for Koran district, while for Azadi district the rates were 27.20%, 6.93%, 26.27%, 14.57% and 10.12% respectively.

[4] indicated the incidence of human intestinal parasites in some villages of Nineveh province, in which several hundred stool samples were collected, examined by both direct and Zinc Sulphate floatation methods. The rate of infection was 40.7%, which includes both protozoa (19.14%) and helminths (21.57%).

[5] has collected 1258 stool samples from children of eight primary schools and foodhandlers in Ninevah governorate. The samples were examined directly and by using the flotation method in Zinc Sulphate and sugar solutions. The total number of infection among schoolchildren was 50.6% of which 29.8% were protozoa, 12.1% helminths and 8.7% mixed infections.

[6] have obtained 461 stool specimens from children resident in day-care centers in Damghan city, Semnan province-Iran. The samples were tested using Formalin-Ether concentration. The analysis of the results showed that at least 68.1% of the individuals tested, were infected with one species of pathogen or non-pathogen parasites. The rate

of infection for *G. lamblia*, *E. histolytica*, *A. lumbricoides*, *H. nana*, *E. coli* and *I. buetschlii* was 26.2%, 2.4%, 3%, 4.8% and 2.7% respectively.

[7] carried out a study to determine the prevalence of parasites in three different regions (Alibaba, Esentepe, and Çayboyu) of Sivas, Turkey. Stool specimens were taken from 1864 participants during 641 household visits in the three regions. The total prevalence of intestinal parasites in the three regions was 37.2%.

A survey was conducted to determine the extent of intestinal parasite infection in Bat Dambang, Cambodia in March 2004. A total of 623 fecal specimens were collected from kindergarten and schoolchildren and examined using the Formalin:Ether sedimentation technique. The overall infection rate of intestinal parasites was 25.7% (boys, 26.2%, girls, 25.1%). The infection rate of *H. nana* was 1.3%, *E. coli* 4.8%, *G. lamblia* 2.9%, *I. buetschlii* 1.4% and *E. histolytica* 0.8%. [8].

The objectives of the present study were to know some information about the prevalence of intestinal parasites among food-handler workers and to compare several methods with respect to accuracy, diagnostic yield, time consumption and cost as well as to investigate and analysis of two extract (*i.e.* Pomegranate molasses as a plant extract and Honey as an animal product), for concentrating ova of helminths and cysts of protozoa on the basis of specific gravity.

Materials and Methods

Pomegranate molasses and Honey sedimentation technique:

The pure ingredients of Pomegranate molasses solution as labeled on its bottle include: Concentrated pomegranate, Sugar, Citric acid, Water and the total volume was 170 ml/bottle.

This solution was diluted by weighing 280gm (210 ml) of concentrated

pomegranate to 1000 ml distilled water, the estimation of its specific gravity was done by using a Baume'-Density-Hydrometer T.P. at 20°C, which was equal to 1.06 gm/cm³, and used for concentration method, prepared Honey solution consists of 100% pure natural Honey, it was diluted by weighing 233gm (166 ml) of Honey in to 1000 ml distilled water then its specific gravity was estimated by using a Baume's-Density-Hydrometer T.P. at 20°C, which was equal to 1.05 gm/cm³, and it was used for sedimentation technique as previously mentioned for pomegranate molasses, then we added approximately 1gm of feces to 10 ml of distilled water, then strained through double layer of wet surgical gauze in to 15 ml centrifuge tubes. The suspension was then centrifuged at 600 rpm for 2 minutes, the supernatant decanted, and the particulate matter resuspended repeatedly (usually 2-4 times) until the supernatant became clear. After an additional decantation the sediment is mixed with Pomegranate solution and the suspension is shaken vigorously for at least 2 minutes, following centrifugation at about 600 rpm for 2 minutes, a thin film of both supernatant and sediment is placed on different slides, and the preparations were mounted with a cover glass for examination.

Zinc Sulphate flotation technique:-

For this method the procedure of (9) was used in order to float ova of helminthes and/or cysts of protozoa. In this method a solution of Zinc Sulphate with a specific gravity of 1.180 gm/cm³ which was prepared by dissolving 331gm of Zinc Sulphate in 1000ml distilled water and adjusting to exact specific gravity using a Baume's-Density-Hydrometer T.P. at 20°C.

Formalin:Ether sedimentation technique:-

Formalin used for fixing and preserving the fecal specimen and ether used to decrease the specific gravity of small fecal particles, causing them to float in the suspension while

the coarser, nonabsorbent elements are left at the bottom, including eggs and cysts of parasites [9, 10].

In this procedure the fecal sample was first comminuted in sufficient distilled water to provide 10-12 ml of suspension, which was strained through 2 layers of surgical gauze into a 15ml centrifuge tube. The suspension was centrifuged at 600 rpm for 2 minutes, the supernatant decanted, and the particulate matter resuspended repeatedly until the supernatant was clear. After an additional decantation, the sediment was mixed with 10 ml of 10% Formalin and allowed to stand for 5 minutes after that 3 ml of ether were added and the suspension shaken vigorously for at least 2 minutes. Following centrifugation at about 600 rpm for 2 minutes, the entire supernatant was poured off. A thin film of the sediment was placed on a slide, a drop of iodine stain was mixed with it, and the preparation was mounted with a cover glass for examination [9].

Statistical analysis:

For evaluating the differences according to different parameters chi-square (α^2) test was used, in which calculated α^2 compared with tabulated α^2 at both levels (0. 01 and 0. 05) of significant.

Results and Discussion

Pomegranate molasses:

From total number of (208) fecal samples were examined by Pomegranate molasses sedimentation method, the total positive cases were 64 cases (30.77%), include both 60 cases (28.85%) of protozoan cysts and only 4 cases (1.92%) of *Hymenolepis nana* ovum. For protozoan cysts were identified 24 cases (11.54%) of *Entamoeba histolytica*, 16 cases (7.69%) of *Entamoeba coli* (Fig. A) and 20 cases (9.62%) of *Giardia lamblia*. were observed, in the same time Pomegranate molasses slightly stained the parasite in the prepared

smear. This method of sedimentation can be considered the first attempt to be used and applied in coprological examination.

The introduction of any new or modified technique should be always including comparisons with the method currently being used [11]. We couldn't compare the results of this method with the results of other authors due to the first application and use of pomegranate molasses solution in this field; therefore as showed in Table (1) and (2) we compared with the other routine methods.

The background quality of smears prepared by the Pomegranate molasses from the sediment of the centrifuged tube was clean and yellow in almost all instances, allowing relatively easy identification of organisms, whereas smears prepared from specimens concentrated with Formalin:Ether method had a dirty background.

Honey:

From total number of (196) fecal samples were examined by Honey sedimentation method, the total positive cases were 52 cases (26.53%), including both 48 cases (24.49%) of protozoan cysts and only 4 cases (2.04%) of *Hymenolepis nana* ovum. For protozoan cysts were identified 12 cases (6.12%) of *Entamoeba histolytica*, 16 cases (8.16%) of *Entamoeba coli* and 20 cases (10.2%) of *Giardia lamblia*. were observed, also this method can be considered the first attempt to be used and applied in coprological examination.

We couldn't compare the results of this method with the results of other authors due to the first application and use of Honey solution in this field; therefore we compared with the other routine methods.

Formalin:Ether

From total number of (372) fecal samples were examined by Formalin:Ether sedimentation method, the total positive cases were 72 cases (19.35%), include both 48 cases (12.9%) of protozoan cysts and 24 cases (6.45%) of helminth ova. For protozoan

cysts were identified 20 cases (5.38 %) of *E. histolytica*, 12 cases (3.23%) of *E. coli* and 16 cases (4.3%) of *G. lamblia*, while for helminthes 24 cases (6.45%) of *H. nana*. were observed.

The higher rates of infection (26.1%) was recorded by [12] in Erbil, but he didn't mention the differences among three methods (Zinc Sulphate, Formalin:Ether and Direct), at the same time he used all above three methods as he mentioned in his materials and methods. The high rates of infection (68.1%) reported by [6] among Iranian population by using Formalin:Ether sedimentation method. The higher rates of infection were recorded by [13] in Erbil (29.6%). These higher differences in rates than the present study could be, as the authors mentioned, due to the low level of education, sanitary conditions, environmental factors and hygiene.

The lower rates of infection (5.22%) recorded by [14] in Sulaimani districts, this lower rate of infection with intestinal parasites than the rate of the present study may be due to the relatively lower temperature in Sulaimani district.

Zinc Sulphate flotation method:-

From total number of (524) fecal samples were examined by Zinc Sulphate flotation method, the total positive cases were 192 cases (36.64%), include both 160 cases (30.53%) of protozoan cysts and 32 case (6.11%) of helminth ova. For protozoan cysts were identified 68 cases (12.98%) of *E. histolytica*, 20 cases (3.82%) of *E. coli*, and 72 cases (13.74%) of *G. lamblia*, while for helminthes 28 cases (5.34%) of *H. nana*, and only 4 case (0.76%) of *A. lumbricoides* were observed.

The higher rates of infection (51.7%) than the present study (36.64 %) was recorded among schoolchildren by (15) in Kirkuk and similar rates of infection (37.2%) was recorded by (5) in Musol and (15) in Kirkuk recorded 35.69% among foodhandlers. (12) in Arbil

were reported lower rates of infection (21.6%) than ours, the later authors mentioned that this due to the high standard of living and better sanitation of the studied groups, and also (14) recorded the lower rates of infection (15.35%) than the present study, he explained the results due to the low temperature degree in Sulaimani district. The higher rates of infection than ours recorded by [4] in Mosul, he recorded 40.7% and mentioned that there were no significant

differences between the direct and Zinc Sulphate flotation method, this may be due to unchecking of floating media for specific gravity by [4]. If Zinc Sulphate is used (should be stored in a tightly-stoppered bottle), the solution must be checked periodically for specific gravity (1.18 for fresh specimens and 1.20 for Formalin-fixed specimens) with a hydrometer whose scale is large enough to differentiate the two values [16].

Table (1): A comparison among three methods (Direct, Floatation, and Sedimentation) of fecal analysis for detection of intestinal parasites of man from number of positive samples.

Parasites	Pomegranate molasses (208)		Honey (196)		Formalin:Ether (372)		Zinc Sulphate (524)	
	No.	%	No.	%	No.	%	No.	%
<i>E. histolytica</i>	24	11.54	12	6.12	20	5.38	68	12.98
<i>E. coli</i>	16	7.69	16	8.16	12	3.23	20	3.82
<i>G. lamblia</i>	20	9.62	20	10.20	16	4.30	72	13.74
Total protozoa	60	28.85	48	24.49	48	12.90	160	30.53
<i>H. nana</i>	4	1.92	4	2.04	24	6.45	28	5.34
<i>A. lumbricoides</i>	0	0	0	0	0	0	4	0.76
Total Helminths	4	1.92	4	2.04	24	6.45	32	6.11
Total Parasites	64	30.77	52	26.53	72	19.35	193	36.64

$$\alpha^2 = 26.38694$$

Table (2): Rates of infection with intestinal parasites of man of (524) examined fecal samples in Erbil-province according to different methods of analysis.

Analysis Methods	Number of examined samples	Number of positive samples	Rate (%) from total of examined samples
Formalin:Ether sedimentation	372	72	19.35
Pomegranate molasses sedimentation	208	64	30.77
Honey sedimentation	196	52	26.53
Zinc Sulphate flotation	524	192	36.64
Total	1300	380	29.23

$$\alpha^2 = 9.16852$$



-A-

-B-

Fig.(A):- Direct-iodine wet mount smear preparation, shows cyst of *Entamoeba coli* (400X).
C: Cyst of *Entamoeba coli*.

Fig.(B):- Smear preparation without iodine (*Entamoeba coli*) from the sediment of Pomegranate molasses (400X).
C: Cyst of *Entamoeba coli*.

References

- [1] Akhtaruzzaman, K.M.; Bienzle, U.; Rosenkaimer, F.; Guggenmoos, R. and Dietrich, M. (1978). Comparison of different methods for the detection of intestinal protozoa and helminths in Stool. *Trop. Med. Parasit.*, 29: 437-431.
- [2] Truant, A.L.; Elliot, S.H.; Kelly, M.T. and Smith, J.H. (1981). Comparison of formalin-ethyl acetate sedimentation, and zinc-sulfate floatation techniques for detection of intestinal parasites. *J. Clin. Microbiol.*, 13 (5): 882-884.
- [3] Molan, A.L. and Farag, A.M. (1989). Prevalence of intestinal parasites in schoolchildren of Arbil-Northern Iraq. *Saud. Med. J.*, 10 (2): 107-110.
- [4] AL-Kachanche, I.M.Y. (1989). Study on the incidence of human intestinal parasites in some villages in Nineveh Province, with study of the effect of some physical and chemical factors on the development and viability of *A. lumbricoides* (Linnaeus, 1758) ova. M.Sc.Thesis. Mosul Univ. Iraq. 139pp.
- [5] Al-Daoudy, A.A. kh. (1998). Epidemiology of intestinal parasites among pupils of a number of primary schools and foodhandlers in Ninevah Governorate. M.Sc. Thesis. Mosul Univ., 117p.
- [6] Heidari, A. and Rokni, M.B. (2003). Prevalence of intestinal parasites among children in Day-Care Centers in Damghan-Iran. *Iranian J. Publ. Hlth.*, 32 (1): 31-34.
- [7] Çeliksöz, A.; Güler, N.; Güler, G.; Öztop, A.Y. and Degerli, S. (2005). Prevalence of intestinal parasites in three socioeconomically-different regions of Sivas, Turk. *J. Hlth. Pop. Nutr.*, 23 (2): 184-191.
- [8] Park, S.K.; Kim, D.H.; Deung, Y.K.; Kim, H.J.; Yang, E.J.; Lim, S.J.; Ryang, Y.S.; Jin, D. and Lee, K.J. (2004). Status of intestinal parasite infections among children in Bat Dambang, Cambodia. *Korean J. Parasitol.*, 42 (4): 201-203.



- [9] Faust, E.C.; Beaver, P.C. and Jung, R.C. (1978). Animal agents and vectors of human disease. 3rd edn. Philadelphia: Lea and Fediger. Chapter 18 (247-269).
- [10] Henry, J.B. (2001). Clinical diagnosis and management by laboratory methods. 12th edn. W. B. Saunders Company, Philadelphia. 1197p.
- [11] Garcia, L.S. and Shimizu, R. (1981). Comparison of clinical results for the use of ethyl acetate and diethyl ether in the formalin-ether sedimentation technique performed on polyvinyl alcohol-preserved specimens. J. Clin. Microbiol., 13 (4): 709-713.
- [12] Kadir, M.A.; Kader, A.A. and Faraj, K.K. (1987). Survey study of the intestinal parasites among different population of Arbil City J. Fac. Med. Bagh., 29 (4): 455-458.
- [13] Farag, A.M. (2000). Prevalence of intestinal parasites in some of Kindergartens in the center of Arbil-Northern Iraq. Dohuk Univ. J., 3 (1): 7-12.
- [14] Hussein, R.H. (2003). Epidemiological study of intestinal parasites among population in Sulaimani District. M.Sc. Thesis. Sulaimani Univ., 99pp.
- [15] AL-Shirifi, H.M.H. (2000). Prevalence of intestinal parasites among pupils of primary schools and Foodhandlers in AL-Taamem province-Iraq. M.Sc. Thesis. Mosul Univ., 180pp.
- [16] College of American Pathologists (CAP). (2004). Commission on laboratory accreditation. 105 pp, 83-90 p.