Breif Communication

Comparison of two concentration techniques in the detection of intestinal parasites

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Abstract: The diagnostic performance of two concentration methods, Tween-80 and Formol-Saline, were evaluated using a total of 400 stool samples from patients referred to the Ethiopian Health and Nutrition Research Institute. Both tests showed similar rates of detection; Formol-Saline (50.3%) and Tween-80 (51%), and no significant difference was observed. The sensitivity and specificity of the Tween-80 method relative to the Formol-Saline technique were 97.0% and 95.5%, respectively. However, from the point of view of the relative availability of reagents and simplicity, the Formol-Saline concentration method is recommended for the diagnosis of intestinal parasites in basic service-giving health institutions and peripheral laboratories. [*Ethiop. J. Health Dev.* 1998;12(2):161-163]

Introduction

The diagnosis of intestinal parasitic infections largely depends on the microscopic examination of stool specimens. It has been noted that various concentration methods have special advantages for the detection of parasites especially when the parasite load is low. There are specific methods that are mainly used for the detection of ova and cysts of the intestinal parasites.

The Formol-Saline technique is considered to be reliable for recovering most of the ova and cyst of the intestinal parasite (1). The Tween-80 method (2) was introduced to the Ethiopian Health and Nutrition Research Institute (EHNRI) Parasitology Laboratory for routine processing of stool for ova and parasites and has been confined only to this Institute. Its potential use in the peripheral service-giving laboratories and health institutions has not to date been evaluated.

The establishment of well equipped laboratories with little or some integration into existing health care facilities is believed to promote efficient diagnosis of intestinal parasites in the community (3). The availability of cheap, safe and effective diagnostic methods is also believed to contribute to the feasibility and sustainability of control strategies of intestinal parasites. The aim of the study was to evaluate the reliability and effectiveness of Tween-80 and Formol-Saline methods for the detection of intestinal parasites.

Methods

Sample collection: A total of 400 fresh stool specimens were collected in labelled stool cups from patients referred to EHNRI for the diagnosis of ova and parasites. From the total stool specimen referred daily, every 10th sample was randomly selected and processed by the two concentration techniques as described below. The results were statistically analyzed using MCNamer test. *The Formol-Saline Method*: All the 400 stool specimens were processed using the Formol-Saline method as described previously (1) with some modifications. Briefly, from each person about

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two grams of fresh stool was taken in a labelled beaker and eight ml of Formol-Saline (900 ml of saline and 100 ml of formalin 37% per litre), PH,7.0 was added and thoroughly mixed using an applicator stick. The mixture was sieved in a labelled test tube using a double fold cotton gauze and funnel. Four ml of diethyl ether (Reidaldien, Chem, USA) was added to the elute. The test tube, fitted with a stopper, was shaken well and centrifuged at 2000 rpm for two minutes. The supernatant

was discarded and the sediment examined for the presence of ova, larvae and cysts of the intestinal parasites using light microscope at 10x and 40x magnifications.

The Tween-80 method: Simultaneously, the same number of stool samples from the same individuals were processed using the Tween-80 technique. Two grams of stool was taken in a labelled beaker containing eight ml of saline and mixed using wooden applicator sticks; then sieved in a labelled test tube through double fold cotton gauze, centrifuged at 2000 rpm for two minutes. To the sediment, seven ml of Tween-80 in citric acid buffer solution and three ml of diethyl ether was added, (with the Tween-80 citric acid solution prepared as follows: citric acid 12.914 gm; disodium hydrogen phosphate, 27.614 gm ; merzoni, 0.1 gm per litre; to 50 ml of the above solution 5ml of Tween- 80 was added, stirred thoroughly). The suspension in a test tube with a rubber stopper was then shaken well, thoroughly mixed and centrifuged at 2000 rpm

Table 1: **referred to EHNRI Parasitology Lab in 1995.Percent positivity of intestinal parasite species identified by the Tween -80 and Formol-Saline methods among stool specimens**

No. Of stool specimens screened and positive				
Parasite species Identified	Tween-80 (N=400)	%Pos	Formol Saline (N=400)	% Pos
Ascaris lumbricoides	43	10.8	43	10.8
Trichuris trichura	76	19.0	79	19.8
Hookworm sp.	18	4.5	16	4.0
Strongyloides stercoralis	20	5.0	17	4.3
Schistosoma mansoni	12	3.0	17	4.3
Fasciola species	4	1.0	3	0.8
Hymenolepis nana	5	1.3	7	1.8
Entrobius vermicularis	-	-	2	0.5
Giardia lamblia cyst	9	2.3	9	2.3
Isospora spp.	3	0.8	2	0.5
Total	204	51%	201	50.3%

for two minutes. The supernatant was discarded and the sediment examined under the light microscope for the presence of ova, larva and cysts of the intestinal parasites at magnifications of 10x and 40x.

Results

Various species of intestinal parasites were identified by their characteristic ova, cyst and larvae using both techniques from the same individuals in each case (Table 1). Except for *Enterobius vermicularis*, which was not detected by Tween-80 method, almost comparable results were obtained by both methods. There was no significant difference in the results obtained by both techniques. The sensitivity and specificity of the Tween-80 method, relative to the Formol-Saline method, were 97.0% and 95.5%, respectively (Table 2).

Table 2: **Parasitology Lab in 1995Comparative parasite detection rate of the Formol -Saline and Tween-80 methods from stool specimens obtained from EHNRI**

Tween-80	Formol-Saline		
	Positive	Negative	Total
Positive	195	9	204
Negative	6	190	196
Total	201	199	400

Discussion

Undoubtly, intestinal parasitism is one of the major public health problems in developing countries, including Ethiopia. Efficient laboratory services become vital for effective diagnosis and control of intestinal parasitic diseases which are rampant in countries like ours. However, the Parasitology Laboratory of the EHNRI, based on the new policy of the EHNRI, is entrusted, among

other things, with referral diagnoses of disease-causing agents at the community level to contribute for the prevention and control of major parasitic diseases, including intestinal parasites.

The microscopic technique of stool examination is the simplest, reliable and most commonly used for the detection of intestinal parasitic infections. This is commonly done either by the direct saline or by the concentration method (3,4). The direct saline method is used to detect live trophozoites and larval stages of intestinal parasites. The movement of motile stage of parasites can no longer be seen otherwise if formalin or ether is

added. However, by employing direct method, ova and cysts of the parasite are frequently missed when they are rare as in light infections as compared to concentration methods (5,6). Lack of a most reliable and easily applicable concentration technique in the hospital and health center laboratories may, therefore, aggravate the problem of diagnosis and control of intestinal parasitic diseases (7).

In this study the effectiveness of the two concentration methods, i.e., the Formol-Saline and Tween-80, in recovering ova, larvae and cysts of intestinal parasites is similar. The chance of being positive or negative for a specimen was also equal in both techniques. Only two cases of *E. vermicularis* were identified by the Tween-80 method, which was missed by the Formol-Saline technique. This does not, however, show a significant difference for the diagnostic capability of the two methods.

Worth mentioning would also be that cysts of *Giardia lamblia* were more often destroyed by the Tween-80 method while found intact without loosing their actual morphology in the Formol-Saline preparation. This could be attributed to the preservative nature of the reagent formalin which is rather more recommended for field work. Eventhough it is not widely used, the Tween-80 method is more comfortable than the Formol-Saline method, since the latter is highly irritating during the preparation and microscopic observation of the stool samples. The Tween-80 reagents, however, are not readily available for wider use irrespective of the technical simplicity compared to the Formol- Saline method. In contrast, the Formol-Saline technique is relatively cheaper and the reagents required are readily available as compared to the chemical ingredients required to prepare the Tween-80 solution. Furthermore the Formol-Saline method has an additional advantage; it is simpler, requires less time and is recommendable as a diagnostic tool for routine purposes and field epidemiological studies of intestinal parasites. It is, therefore, suggested that the servicegiving health institutions and peripheral laboratories make use of the Formol-Saline technique to improve their diagnostic capabilities than to depend more on the classical direct saline method.

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References

1. Cheesbrough M. Medical laboratory Manual for Tropical Countries. 1981;1:139-142.
2. Oshima T, Kagel Kihata M, Fujino N, Noguchi H, Fujioka K. A new effective ether sedimentation procedure for the recovery of the eggs of chonorchis sinensis and Metagonimus Yoko gawal in the stools. J. of Parasitol. 1965;14(2):195-203.
3. Engels D, Nahimana S, Grysees B, Comparison of the direct faecal smear and two thick techniques for the diagnosis of intestinal parasitic infections. Trans Roy Soc Trop Med Hyg. 1996;90:523-525.
4. World Health Organization. Prevention and Control of Intestinal parasitic Infections. Tech Rep Ser. 1987:749.
5. Beaver PC, Jung RC, Cupp EW. Clinical parasitology. (9th edition) Lea and Febiger, Philadelphia. 1984:734.
6. Duncan J, Lemma A, Mazengia BA comparison of certain stool examination techniques with special reference to their efficacy in surveys for schistosomiasis in the Awash valley. Ethiop Med J. 1970;8:17-25.
7. Lennart F. Observation on parasitic and Bacterial infection among paediatric out patients in Addis Ababa. Ethiop Med J. 1973;(11):13-14.