Formol-petrol stool concentration method (Wirkom-Tata's stool concentration method): A Cheap Novel Technique For Detecting Intestinal Parasites In Resource-Limited Countries

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Citation

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Abstract

In search of a new method for stool concentration and to establish the incidence of intestinal parasites infection amongst primary school pupils in Djottin, Cameroon, 477 stool samples were examined in the Medical Laboratory of ST. Elizabeth's Catholic Mission Shisong. Formol-ether concentration technique detected 35.76% of the total number of parasites seen. Formol-petrol concentration method was 34.50% effective, direct smear method was 27.20% effective while the saturated sodium chloride (flotation) method detected only 2.27% of the parasites. The high incidence of intestinal parasite infection in these children was found to be linked to poor hygiene and low level of education of the pupils. The Formol- petrol stool concentration technique which gave a parasite recovery rate of 34.50% which indicates that, it is a new and cheap and very effective method for examination of human stool samples.

INTRODUCTION

Intestinal parasitic infections remain a health problem especially in the developing countries of the world [6]. It should be noted that most of these infections are as a result of low standard of living, poverty, poor sanitation and lack efficient diagnostic facilities.

According to World Health Organisation [11] human intestinal parasites cause significant morbidity and mortality through out the world, particularly in under developed countries and especially in children. Infection by soil-transmitted helminths (intestinal worms) has been increasingly unrecognized as an important public health problem. In the 1993 World Development Report, intestinal parasite ranked first as the main cause of disease burden in children aged 5-14 years and also ranked highly as the disease that can be efficiently controlled by cost- effective intervention.

In order to diagnose intestinal parasites, many methods can be considered. The choice of a particular technique will depend on its affordability, ease to carry out, its effectiveness and level of professionalism involved. Examples of these methods are DNA probes, PCR and direct fluorescent antibody methods [8], which are highly sensitive but are too costly to be used in the developing countries. It has been proved that direct stool smear, formol-ether and salt flotation techniques in stool microscopy offers many advantages over other diagnostic methods of detecting intestinal parasites. If performed correctly, these methods are sensitive, simple and economical [8].

Direct stool smear [4], formol-ether concentration [1] and flotation methods are used in diagnosing intestinal parasites by hospital and researchers in developing countries because of their affordability, simplicity and sensitivity.

For the fact that the density of the parasite in the faeces is low, direct smear method is useful for the observation of motile protozoan trophozites and examination of exudates, but is not recommended solely for the routine examination of suspected parasitic infections [2]. Therefore there is need for increase probability of finding the parasite in the faecal samples to allow for accurate diagnosis, hence there is need

to concentrate. Though direct stool smear technique is quick to prepare and inexpensive when compared with modified formol-ether concentration methods, it can miss parasites (ova, cysts and larvae) if concentration is too low or if too much debris or fat is present. Sand, seeds or other faecal debris can make fixing cover slips onto slide difficulty. Most diagnostic centers show high performance to this method, owing to the fact that it is inexpensive and non-time –consuming thereby disregarding the consequences of misdiagnosis, which has led to prevalence of parasitic diseases and morbid conditions. Such complications resulting from misdiagnosis have been described [3].

Formol-ether concerntration techniques described by Allen and Ridley employs the use of ether or ethyl acetate as a lipid solvent and 10% formol saline as a fixative.This method recovers must ova,cysts and larvae and maintain their structure. In a recent study by Oguoma and E. Kwunife [7] ether concentration technique is a very effective means of stool examination followed by the direct stool smear method. However formol-ether concentration technique is not used by many laboratories due to the expensive nature of ether or ethyl-acetate.

It is then very necessary to look for an effective and cheaper method of stool concentration for the effective diagnosis of intestinal parasites. This study is therefore aimed at comparing three traditional methods of stool examination, direct stool smear, formol-ether concentration or saturated sodium (floatation) techniques with a novel formol-petrol technique. This novel technique makes use of petrol (super) as a fat solvent which is much cheaper than ether.

MATERIALS AND METHODS

This work is based on the examination of stool samples from pupils aged 1-8 years in the primary and nursery schools of Djottin, Cameroon.

STUDY AREA

The study was carried out in Djottin, Noni Sub Division, Cameroon. Though there are many primary schools in Noni sub Division, samples were collected from pupils in seven primary schools out of the total of nine found in Djottin village. These are Catholic nursery schools (CNS) Djottin, Catholic School (CS) Djottin, Government school (GS) Meyessi I-Djottin, Government School (GS) Gaggi, Government School (GS) Bongi, Government School (GS) Champkfung and Government School (GS) Meyessi-II Chieti. This area was chosen for the study because of its warm climate, rural setting, poor hygiene and sanitation which were believed to favour the prevalence of intestinal parasites, especially the nursery and primary school pupils who are lacking in knowledge of personal hygiene.

SAMPLING TECHNIQUES

Stool samples were collected from 477 pupils from the various primary and nursery schools chosen for the study. The names of the pupils to give the stool samples were randomly picked without bias from the school register which were made available to the researcher by the head teachers. Early stool samples were put into scrupulously cleaned sample bottles which were taken to ST Elizabeth's Catholic Mission General Hospital Shisong for processing and examination. Permission to collect samples was taken from the primary school pupils, their teachers and their parents.

PARASITOGICAL TECHNIQUE

The method adopted for direct smear formol-ether concentration and floatation techniques were supplied by WHO [10] and that described by Ukaga et al [9].

FORMOL-PETROL CONCENTRATION TECHNIQUE(DEVELOPED BY VK WIRKOM AND EF TATA)

1 gram of stool sample was emulsified in 10mls of normal saline in a centrifuge tube and spun at 3.000 r.p.m for 10 minutes. The supernatant was discarded. This process was done two times to wash the stool sample. Then the sediment was resuspended in 7mls of formol saline and 3mls of petrol (super) was added and the mixture stoppered with a rubber bung and shaken vigorously. It was spun at 3.000 r.p.m for 10 minutes. It separated into three portions. The first from the bottom was the sediment, followed by a layer of formol saline in the middle and at the top was coarse stool particles, petrol and fats. The layers above the sediment were carefully aspirated and discarded using pasture pipette. The sediment was examined under the microscope for the presence of parasites.

IDENTIFICATION OF INTESTINAL PARASITES

The parasites were confirmed and identified by medical laboratory scientists in accordance with the bench aid for diagnosis of intestinal parasites [6].

STATISTICAL ANALYSIS

The data obtained from the examination of the stool samples and other aspects of this study were statistically analysed using the students T-Test. This helped to establish whether

the efficacy of intestinal parasites diagnosis is dependent on the techniques used and if other factors such as sex, educational level, hygiene and staffing of the schools are related to the prevalence of intestinal parasites.

RESULTS

A total of 447 stool samples collected at random from pupils in six different schools in Djottin were examined for intestinal parasites using formol-ether, formol-petrol, and direct smear and floatation techniques. 368(35.76%) of parasites were detected using the formol-ether concentration, 355(35.5%) by formol-petrol, 278(27.02%) by direct smear and 28(2.72%) by floatation techniques (table 1). All the different types of the intestinal parasites detected by the various methods are shown in table 1.

In table 2, 214(49.77) female pupils tested positive for intestinal parasites as compared to 216(50.23%) male pupils. However, 347(80.70%) of junior pupils(nursery I to class IV) carried intestinal parasites while only 83(19.30%) of the senior pupils (class V-VI) were positive.

It was observed that Catholic Nursery school Djottin accounted for 49(11.40%) of the parasites detected, Catholic school Djottin, 105(24.42%), Catholic school Meyessi I Djottin, 87(20.23%), Government school Bongi, 87(20.22%), Government school Chamkfung, 47(10.93%), Government school Meyessi II Chieti, 40(9.30%) (table 5).

The staffing and toilets facilities in the various primary schools were shown in table 4. It was discovered that averagely, there were 44 pupils per teacher and 67 pupils per toilet facility, The level of hygiene in the homes around the schools indicated that out of 185 homes inspected, 119(64.32%) had access to good source of drinking water,154(83.24%) had toilets while only 17(9.19%) had toilets with lids, 86(46.69%) had human(children) excreta around them, 91(49.92%) had human (children) excreta around fruit trees and 146(78.92%) had animal excreta around homes or fruits trees (table 5).

Figure 1

Table 1: Number and percentage of different parasites found

Intestinal parasite	Formol-ether conc.no. (%)	Formol-petrol conc. no. (%)	Direct smear, no. (%)	Salt floatation method no. (%)	Over all no. (%)
A. lumbricroides	225(21.87)	212(20.60)	192(18.66)	27(2.62)	656(63.75)
T. trichiura	7(0.68)	8 (0.78)	1(0.10)	0(0.00)	16(1.55)
E. vermicularis	1 (0.10)	0 (0.00)	0(0.00)	0(0.00)	1(0.10)
T. saginata	1(0.10)	1 (0.10)	0(0.00)	0(0.00)	2(0.19)
H. nana	0 (0)	1 (0.10)	0(0.00)	0(0.00)	1(0.10)
A. duodenale	1(0.10)	0 (0.00)	0(0.00)	1(0.10)	2(0.19)
F. hepatica	0(0.00)	1 (0.10)	0(0.00)	0(0.00)	1(0.10)
S. stercolaris	1(0.10)	0 (0.00)	2(0.19)	0(0.00)	3(0.29)
E. histolytica	85(8.26)	86 (8.36)	49(4.76)	0(0.00)	220(21.35)
G. lamblia	2 (0.19)	2 (0.19)	1(0.10)	0(0.00)	5(0.49)
Entamoeba coli	41 (3.98)	39 (3.79)	31(3.01	0(0.00)	111(0.79)
I. buetschlii	4 (0.39)	5 (0.49)	2(0.19)	0(0.00)	11(1.07)
TOTAL	**368(35.76)**	**355 (34.50)**	**278(27.02)**	**28(2.72)**	**1029(100)**

Table 1 shows that there is no significant difference ($p > 0.05$) between the parasites detected by formol-ether and formol-petrol concentration techniques. However there was a significant difference ($p < 0.05$) between the results of salt floatation technique as compared with the other 3 methods.

Figure 2

Table 2: Distribution of parasites with respect to sex and educational level of the pupils

Intestinal parasite	Female pupils (%)	Male pupils (%)	Junior section nursery 1-class IV (%)	Senior section class V – VI (%)
massl	126(26.42)	122 (28.37)	201(42.14)	47(9.85)
T. trichiura	5(1.05)	3 (0.63)	6(1.26)	2(0.42)
E. vermicularis	1 (0.21)	0 (0.00)	1(0.21)	0(0.00)
T. saginata	1(0.21)	0 (0.00)	0(0.00)	1(0.21)
H. nana	0 (0.00)	1 (0.21)	0(0.00)	1(0.21)
A. duodenale	1(0.21)	1 (0.21)	1(0.42)	0(0.00)
F. hepatica	1(0.21)	0 (0.00)	1(0.21)	0(0.00)
S. stercolaris	1(0.21)	1 (0.21)	2(0.42)	0(0.00)
E. histolytica	47(9.85)	57 (11.95)	84(17.61)	20(4.19)
G. lamblia	2 (0.42)	2 (0.42)	3(0.63)	1(0.21)
Entamoeba coli	28 (5.87)	25 (5.24)	43(9.01)	10(2.10)
I. buetschlii	1 (0.21)	4 (0.84)	4(0.84)	1(0.21)
Total no. infected	**214**	**216**	**347**	**83**
% incidence	**49.77**	**50.23**	**80.70**	**19.30**

Table 2: Shows that there is no significant sex-linked difference ($p > 0.05$) in the incidence of intestinal parasites in pupils but there is a significant difference

($p < 0.05$) between junior and senior pupils.

Figure 3

Table 3: Distribution of parasites in the various primary schools sampled

Intestinal parasite	C.N.S Djottin	C.S Djottin	C.S Meyessi I Djottin	G.S Bongi	G.S Champkfung	G.S Gaggi	G.S Meyessi II Chieti
A.lumbricoides	24	66	55	30	25	25	23
T. trichiura	0	2	2	1	0	0	3
E. vermicularis	0	0	1	0	0	0	0
T. saginata	1	0	0	0	0	0	0
H. nana	0	0	0	1	0	0	0
A. duodenale	0	0	0	1	1	0	0
F. hepatica	1	0	0	0	0	0	0
S. stercolaris	0	0	0	1	1	0	0
E. histolytica	14	26	17	14	13	14	6
G. lamblia	3	0	0	0	1	0	0
Entamoeba coli	5	10	12	4	5	9	8
I. buetschlii	1	1	0	2	1	0	0
Total	**49**	**105**	**87**	**54**	**47**	**48**	**40**
% incidence	**11.40**	**24.42**	**20.23**	**12.56**	**10.93**	**11.16**	**9.30**

Table 3: Shows that the incidence of intestinal parasites is common in all the schools with the highest incidence in catholic school Djottin 105 (24.42%) while the least is in government school Meyessi II Chieti 40 (9.30%).

Figure 4

Table 4: Staff and toilet facilities in the schools

Schools	Total enrolment of pupils	Total no. of toilet squatting spots	No. of teachers	No. of pupils per teacher	No. of pupils per toilet squatting spot
C.N.S Djottin	87	2	3	29	44
C.S Djottin	291	6	6	49	49
G.S Meyessi I Djottin	85	3	3	28	28
G.S Bongi	141	4	4	35	35
G.S Gaggi	260	2	5	52	130
G.S Champkfung	295	1	4	74	295
G.S Meyessi II Chieti	120	1	4	30	120
Total / Average	**1279**	**19**	**29**	**44**	**67**

table 4: Shows that staff and toilet facilities are limited in all the schools studied with an average of 44 pupils per teacher and 67 pupils per toilet squatting spot.

Figure 5

Table 5: Level of hygiene of homes around the schools

Schools	Total number of homes around the school inspected	Presence of good source of drinking water	Presence of toilets	Presence of toilets with lids	Presence of rat-proof toilets	Presence of human (children) faeces around home	Presence of human (children) faeces around fruit trees	Presence of animal faeces around home or fruit trees
	N°	**N° (%)**	**N° (%)**	**N° (%)**	**N° (%)**	**N° (%)**	**N° (%)**	**N° (%)**
C.N.S Djottin & C.S Djottin	31	25 (80.84)	21 (67.74)	3 (9.17)	4 (12.90)	13 (41.94)	19 (61.29)	24 (77.42)
G.S Meyessi I Djottin	35	1 (2.86)	29 (82.86)	3 (8.57)	0 (0.00)	19 (54.29)	19 (54.29)	30 (85.71)
G.S Bongi	20	16 (80.00)	17 (85.00)	3 (15.00)	2 (10.00)	9 (45.00)	9 (48.00)	13 (65.00)
G.S Gaggi	19	16 (84.21)	17 (89.47)	1 (5.26)	0 (0.00)	9 (47.37)	7 (36.84)	8 (42.10)
G.S Chamkung II	36	34 (94.44)	34 (94.44)	1 (2.77)	1 (2.78)	15 (41.67)	15 (41.67)	31 (86.11)
G.S Meyessi II Chieti	44	27 (61.36	36 (81.82)	6 (13.63)	6 (13.64)	21 (47.73)	22 (50.00)	40 (90.91)
TOTAL	**185**	**119 (64.30)**	**154 (83.84)**	**17 (9.19)**	**13 (7.02)**	**86 (46.49)**	**91 (49.19)**	**146 (78.92)**

Table 5: Shows the level of hygiene in homes around the schools where the pupils live which is generally poor

DISCUSSION

Lack of efficient diagnostic facilities and properly trained laboratory personnels remain major issues that fuel the persistence of intestinal parasites especially in poor countries of the world. It is in this light that this study included a new and cheap method, Formol-petrol stool concentration technique (Wirkom-Tata's stool concentration method) to draw the attention of the health sector in developing countries to an alternative way of achieving effective stool examination at an affordable cost. The parasite recovery rate of 34.50 % for formol-petrol method as compared to 35.76% for formol-ether, 27.02% for direct smear and 2.72% for salt floatation methods, is high enough to be regarded as one of the best methods for stool examination in terms of its effectiveness and cost. This result agrees favourably with that of Oguoma and Ekwunife [7], who recorded an intestinal parasites recovery rate of 65.26% for formol-ether and 34.74% for direct smear methods which clearly indicated the superiority of the formol-ether technique. Petrol conveniently replaces formol-ether in stool concentration technique because it is also a fat solvent and equally has a low density. Even though both ether and petrol have their disadvantages of being highly inflammable with irritant odours, proper care in handling them can ensure substantial safety.

From this study the parasites with the highest incidence were Ascaris lumbricoides 63.75%, Entamoeba histolytica 21.38 %, Entameoba coli 10.79% and Trichuris trichiura 1.55%.These parasites can all be easily eradicated with improvement in personal hygiene, enviormental sanitation

and availababilty of potable drinking water [11]. The intestinal parasitic incidence of 80.70% in junior primary school pupils as opposed only 19.30% in the senior pupils shows that advance knowledge in personal hygiene that comes with advancement in education can play a major role in education of infections. Although there was no significant sex-link difference in the incidence of intestinal parasites, a slightly higher incidence of 50.23% for boys as compared to 49.77% for girls is understandable as boys are more prone to risky behaviours than girls.

Averagely in this locality there are 29 pupils per teacher and 67 pupils have to cue up to use one toilet squatting spot. This lack of teaching and toilet facilities are of major concerns if these parasitic infections are to be controlled. According to Kucik et al [5], overcrowding and poor sanitation contribute to the prevalence intestinal parasites in Asia, Africa and Latin America. During home inspection in Djottin, it was discovered that sanitation in many homes is poor. Some homes do not have access to portable drinking water while some do not even have toilets. Majority of home that have toilets, do not cover them with lids. There were human and animal excreta around homes and fruit trees. These are all major factors that can promote the high prevalence and spread of intestinal parasites. This explains the high prevalence rate of intestinal parasites amongst children of this locality.

CONCLUSION

There is serious need to continue emphasis and sensitization on good personal and environmental hygiene as means of preventing the persistence parasitic infections in developing countries. This should be achieved through proper education of the children and adults. In this light, the problem of lack of teachers in the primary schools in less developed countries must also be combated effectively.

Also the lack of well trained health personnel for diagnosis and treating intestinal parasite infections is a serious problem that needs to well handled . The high cost of proper stool examination using the concentration method is one of the factors hindering proper diagnosis, which this research addresses by introducing a cheaper and effective method.

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