

RESEARCH ARTICLE

PERFORMANCE EVALUATION OF DIRECT WET MOUNT TECHNIQUE WITH RESPECT TO FORMOL ETHER CONCENTRATION TECHNIQUE, AT THE BAMENDA REGIONAL HOSPITAL

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ABSTRACT: Intestinal parasites are neglected tropical diseases which still cause tremendous morbidity, despite recommendations for massive de worming by the world health organization. The direct wet mount technique has over the years been used as the routine method for the diagnosis of intestinal parasites in the current study area. Consequently, it is essential to evaluate its diagnostic performance in relation to a more sensitive but rarely used technique. This study was therefore designed to evaluate the performance of wet mount technique with respect to the formol ether concentration technique. Out of the 212 examined patients, 06.60% (14/212) and 16.98% (36/212) tested positive by direct wet mount and formol ether concentration techniques respectively. The following intestinal parasites were diagnosed; *Gardia lamblia*, *Entamoeba coli*, *Entamoebahistolytica*, *Isosporabustchli*, *Fasciola hepatica*, *Enterobiusvermicularis* and *Hookworm*. There was a significant [P=0.0424] difference between the test results by both techniques. Thekappa values between both techniques was lower for the diagnosis of helminthic than for protozoan infections. Therefore, the formol ether concentration technique should be used to complement the routine direct wet mount technique in the study area.

KEYWORDS: Direct wet mount, Formol ether concentration, Intestinal parasites, Performance evaluation

INTRODUCTION:

Intestinal parasites considered as one of the neglected tropical diseases greatly affects those living in developing countries^[1]. The occurrence of intestinal parasites vary globally according to geographical location^[2,3]. For so many years the formol ether concentration (FEC) and direct wet mount techniques are used as means to diagnose intestinal parasites^[4]. However the direct wet mount technique

is poorly sensitive in the diagnosis of less severe infections^[5, 6], while the FEC technique is more sensitive^[5,7]. Although the direct wet mount technique has been shown to have poor sensitivity, it is cheap, time saving, easy and fast to perform^[8]. Consequently the direct wet mount technique remains the routine diagnostic method for helminthic and protozoal infections in Cameroon in particular and in Africa in

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general^[6, 9, 10]. Other sensitive methods which include the kato-katz technique, antigen/antibody assays, FLOTAC and PCR, are not routinely used because of cost, inaccessibility and low sensitivity in low infections^[7, 8, 11-18]. On the other hand, the FEC technique was not only found to be sensitive, but also accessible^[19]. Because the wet mount technique is routinely used in the study area, it is necessary to understand its diagnostic challenges with respect to the more sensitive and cost effective technique, the FEC. Therefore this study was designed to evaluate the performance of the direct wet mount technique with respect to the FEC technique.

Background of Study

Studies have continuously demonstrated that intestinal parasitosis is an ongoing public health problem especially in the developing countries. In India and Tanzania, the most commonly diagnosed intestinal parasites were hookworm, *Entamoeba histolytica/dispar*, *Entamoeba coli*, *Giardia intestinalis* [and *Schistosoma mansoni*, in Tanzania]^[16]. The prevalence of intestinal helminths by direct wet mount and FEC was also reported to be 18.8% (70/372) and 24.7% (92/372) respectively^[20]. In that study, the kappa value for the detection of Hook worm and *Ascaris lumbricoides* was 0.81, and 0.39^[20]. In another study, the sensitivities of wet mount, FEC and kato-katz were 61.1%, 92.3% and 58.7%, respectively^[19]. A study which rather evaluated formalin-gasoline technique in the concentration of stool for detection of intestinal parasites demonstrated that gasoline and ether could both concentrate parasite egg and cysts well, while preserving their distinctive morphology^[6]. Formalin-tween (FTC), formalin-ether (FEC), formalin-acetone (FAC) and formalin-gasoline concentration [FGC] techniques were evaluated for the diagnosis of intestinal parasites. Although FTC and FAC techniques were more sensitive for the diagnosis of helminth ova, FEC and FGC were more sensitive for the diagnosis of protozoan cysts. The κ value showed lower agreement for the diagnosis of helminths than

for protozoa^[21]. Formalin-ether method was also compared with formalin-acetone sedimentation and there were no significant difference in the parasite recovery rate between both techniques^[22].

Entamoeba histolytica and *Ascaris lumbricoides* were found to be most prevalent [65.57%] and modified formol-ether sedimentation technique indicated a high sensitivity for the detection of intestinal parasites^[23]. Another study reported the prevalence of intestinal parasite as 39.2%, meanwhile the sensitivities of formol ether and direct smear were found to be 75% and 65% respectively^[24]. Moreover, in the diagnosis of *Schistosoma mansoni* infections in areas of high (63.3%) and low prevalence (40%), the sensitivity of the formol ether sedimentation technique was found to be 83.3%^[13]. Infant geohelminths were evaluated using the Wisconsin floatation/simple gravity and the sedimentation Kato-Katz technique/simple gravity sedimentation which both had highest sensitivity (99.0%) among other methods tested^[25]. Likewise a study evaluated the diagnostic performance of direct, formol ether concentration (FEC) and Kato Katz methods using the combined results as "gold standard". The prevalence of intestinal parasites was 74.6%^[26]. Although the kato-katz technique outperformed the other two techniques, it was considered labor intensive and inaccessible. Therefore the FEC technique was recommended as a complement for the wet mount technique^[26].

The combined four diagnostic methods were considered as fixed "gold" standard and the prevalence of intestinal parasites was 36.2%, the kato-katz technique had the best performance^[8]. In that study the sensitivities of Kato-Katz technique (KK), the spontaneous sedimentation technique in tube (SSTT), the formalin-ether concentration technique (FEC), and direct smear microscopy (DM), were 71%, 58%, 50% and 3% respectively^[8]. Another study which evaluated the performance of wet mount, formol-ether concentration (FEC) and Kato-Katz techniques also used the combined three techniques

as “gold” standard. Parasite prevalence by single wet mount, FEC, and Kato-Katz thick smear techniques were 38.4%, 57.1%, and 59%, respectively^[27]. The sensitivities of FEC and Kato-Katz thick smear techniques were 81.0% and 78.3% respectively, meanwhile their negative predictive values were 66.2% and 63.2% respectively^[27]. For the diagnosis of hookworm and *Ascarislumbricoides*, the k agreement between the kato-katz and wet mount techniques were 0.65 and 0.61 respectively^[27].

Where the Kato-katz and ether concentration techniques were compared, quadruplicate kato-katz had a higher sensitivity at baseline and follow-up. But the ether concentration technique showed a higher sensitivity for the diagnosis of *Ascarislumbricoides* and for all soil transmitted helminths (STH) infections based on a single stool sample^[28]. The formol-ether and kato-katz techniques were equally evaluated in another study and results showed that, although the kato-katz method is the most recommended, the concentration technique had higher sensitivities in the diagnosis of each types of helminths^[29]. In the identification of *Sarcocystis* infection the modified Kato-katz technique was shown to be more sensitive than the direct smear procedure^[14]. In that study the following intestinal parasites were found; hookworm (10.2%), *Taeniaspp* (2.9%), *Strongyloidesstercoralis* (2.1%), *Giardia lamblia* (1.2%), *Ascarislumbricoides* (0.4%) and *Entamoeba histolytica* (0.2%) among others^[14]. Further more the operational characteristics of wet mount, formol ether and kato-katz techniques were evaluated. There was a substantial agreement between the kato-katz and wet mount techniques in the diagnosis of hookworm and *Ascarislumbricoides*^[27].

Finally, the FLOTAC technique has also been widely investigated in the diagnosis of intestinal parasites. The diagnostic accuracy of four copromicroscopic techniques were evaluated for the detection of *Schistosoma mansoni* and soil-transmitted helminth eggs. Although the FLOTAC technique had the

highest sensitivity, it resulted in lower egg counts^[30]. The FLOTAC technique was shown to be more sensitive than the Kato-katz and ether concentration techniques in the diagnosis of hookworm infections^[31]. Considering their combined results as “gold” standard, the sensitivities of the FLOTAC, kato-katz and ether concentration techniques were 88.2%, 68.4% and 38.2%^[31]. Contrary to this, the formol ether concentration method has also been reported to be effective and widely used^[32].

MATERIALS AND METHODS:

Study area and population

This study was carried out in the Bamenda Regional Hospital [BRH], which is the main government hospital in the North West Region of Cameroon. Bamenda is located along 10.15 longitude and 5.96 latitude and is also situated at the height of 1258 meters above the sea level. There are two seasons in Bamenda, the dry and the rainy seasons, with a balance rainfall per year being 2064mm [and 172mm per month]. The peak of dry season occurs in January, meanwhile the peak of rainy season is in September. The BRH is part of the Bamenda Health District [BHD], which is made up of many public, private and mission health facilities located within the 17 health areas in the BHD. The BRH therefore operates as the referral hospital in the region, with an estimated 337,036 inhabitants^[33].

Study period/participants

This study was carried out within the period of three months, from February 2019 to April 2019. The study population was all the patients consulting at the BRH and who were sent to the laboratory for stool analysis during the study period. All those who gave their consent [or “assent” for children] by signing the informed consent forms [or “assent forms”] were enrolled into the study. A total of 212 patients who were enrolled into the study were given sterile stool cups and properly instructed on how to collect the stool samples. 212 stool samples were collected from

the 212 study participants. The ethical clearance for this study was gotten from the Ethical Review Committee of the University of Bamenda.

Laboratory diagnostic methods

- **Direct wet mount technique**

For diarrheic and semi solid stool samples, a wooden applicator was use to transfer about 2mg of fresh stool sample on a clean slide and emulsified with a drop of 0.85% physiological saline [iodine was used for formed stool]. The preparation was covered with a cover slide and examined under 10X objective first and then 40X objective^[24].

- **Formol ether concentration technique(FEC)**

0.5g of stool sample was thoroughly mixed in 10ml of normal saline, in a glass container. The contents were strained into a 15ml centrifuge tube through two layers of gauze, which were placed in the funnel. 1ml of ether and 2.5ml of 10% formaldehyde was added to the filtrate and properly mixed and centrifuge at 1000 revolution for 3 minutes. The supernatant was discarded and the sediment used to make wet mounts preparations with both normal saline and iodine, on two separate slides. Cover slides were placed on these preparations and examined microscopically. The total number of parasitic eggs/cysts counted were multiplied by two to get the number of parasites per gram of stool. The intensity of infection was defined as mild [1 to 100 parasites per gram of stool], moderate (101 to 400 parasites per gram of stool) and heavy (> 400 parasites per gram of stool)^[24].

- **Data Analysis**

Baseline characteristics and paired t-test of patients with and without intestinal parasites were determined using the statistical package Graph Pad prism version 8.2.1. A four-fold (2x2) contingency table displaying the frequency distribution for each intestinal parasite was entered into GraphPad Prism. Chi-square (and

Fisher's exact) test was used to calculate sensitivity (%), specificity (%) and predictive values (%). The level of reliability of the results were determined by the confidence interval, which in this study was at 95%.

RESULTS:

Table 1 shows the distribution of intestinal parasites according to age and sex, by the FEC technique. A total of 16.98% (36/212) of the study participants tested positive for a single intestinal parasite. Parasite load were mild for all the positive cases. Most (49.06%; 104/212) of the study participants were within the 20-30 years age group. This age group also had the highest percentage (44.44%; 16/36) of those who tested positive for intestinal parasites. The female: male ratio was 1.65. 77.78% (28/36) of those infected were females.

Table 1. The distribution of intestinal parasites according to age and sex

Age range (yrs.)	Positive by FEC (%)	Negative (%)	Frequency (%)
	N=36(16.98)	N=176(83.02)	N=212
≤19	06(12.50)	22 (13.41)	28(13.21)
20-30	16(44.44)	88(50.00)	104(49.06)
31-40	02(04.17)	40(24.39)	42(19.81)
41-50	02(04.17)	08(04.88)	10(04.72)
>51	10(20.83)	18 (10.98)	28(13.21)
sex			
male	8(22.22)	72(40.91)	80(37.74)
female	28(77.78)	104(59.09)	132(62.26)

Source: Calculated by authors

The distribution of intestinal parasites identified in each diagnostic test is shown on table 2 below. Out of a total of 212 patients who were examined, 06.60% (14/212) tested positive by wet mount technique and 16.98% (36/212) tested positive by FEC techniques. The test agreement between the wet mount and FEC for the diagnosis of *G. lamblia*, *E. coli*, and *E. histolytica* was excellent (K=1), good (K = 0.75) and

poor (K=0.375) respectively. The test agreement for the diagnosis of *I. bustchli*, *F. hepatica*, *E. vermicularis* and *Hookworm* were all very poor (K = 0). Generally, the kappa value (K =0.3889) is poor for the test agreement between the wet mount and FEC techniques. There is a significant difference (P =0.0424) between the test results of the direct wet mount and the FEC techniques.

Table 2. The ability of the direct wet mount technique to detect intestinal parasites against the FEC technique

	Total Examined	Wet Mount Positive (N, %)	Wet mount Negative (N, %)	FEC Positive (N, %)	FEC Negative (N, %)	Kappa value (%)	Kappa value for both techniques (%)	Paired t test P value
<i>E. coli</i>	212	6 (02.83%)	206 (97.17)	8 (03.77%)	204 (96.24)	0.75 (75%)		
<i>E. histolytica</i>	212	6 (02.83%)	206 (97.17)	16 (07.55%)	196 (92.45)	0.375 (37.5%)		
<i>G. lamblia</i>	212	2(0.94%)	210 (99.06)	2 (0.94%)	210 (99.06)	1 (100%)		
<i>I. bustchli</i>	212	0 (0%)	212 (100)	2 (0.94%)	210 (99.06)	0	0.3889 (38.89)	0.0424
<i>F. hepatica</i>	212	0 (0%)	212 (100)	2 (0.94%)	210 (99.06)	0		
<i>E. vermicularis</i>	212	0 (0%)	212 (100)	2 (0.94%)	210 (99.06)	0		
<i>Hookworm</i>	212	0 (0%)	212 (100)	4 (1.89%)	208 (98.11)	0		

Source: Calculated by authors

With 95% confidence interval, the sensitivity, specificity, positive and negative predictive values, were estimated for the wet mount technique, using the FEC technique as a “gold” standard. The specificities and positive predictive values (PPVs) of the wet mount technique in diagnosing the intestinal parasites were all 100%. However, the sensitivities varied according to the type of parasite. The wet mount technique was most sensitive (100%) in the diagnosis of *G. lamblia* and least sensitive (50%) in

the diagnosis of *I. bustchli*, *F. hepatica*, *E. vermicularis* and *Hookworm*.

Table 3. The performance of direct wet mount diagnostic technique with the FEC technique as “gold” standard.

Variables	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV (%) (95% CI)	NPV (%) (95% CI)	P value
<i>E. coli</i>	80 (49.02 to 96.45)	100 (98.15 to 100)	100 (67.56 to 100)	99.03 (96.53 to 99.83)	<0.0001
<i>E. histolytica</i>	61.54 (42.53 to 77.57)	100 (98.08 to 100)	100 (80.64 to 100)	95.15 (91.30 to 97.34)	<0.0001
<i>G. lamblia</i>	100 (17.77 to 100)	100 (98.20 to 100)	100 (17.77 to 100)	100 (98.20 to 100)	<0.0001
<i>I. bustchli</i>	50 (88.84 to 91.12)	100 (98.20 to 100)	100 (17.77 to 100)	99.06 (96.63 to 99.83)	0.0003
<i>F. hepatica</i>	50 (88.84 to 91.12)	100 (98.20 to 100)	100 (17.77 to 100)	99.06 (96.63 to 99.83)	0.0003
<i>E. vermicularis</i>	50 (88.84 to 91.12)	100 (98.20 to 100)	100 (17.77 to 100)	99.06 (96.63 to 99.83)	0.0003
<i>Hookworm</i>	50 (21.52 to 78.48)	100 (98.19 to 100)	100 (51.01 to 100)	98.11 (95.25 to 99.26)	<0.0001

Source: Calculated by author using Graph pad prism

INTERPRETATION AND DISCUSSION:

The prevalence of intestinal parasites in this study by the FEC technique was 16.98% (36/212) which can be considered low. Moreover there were no cases of co-infections since all the registered positive cases were single infections. A similar finding was recorded among pregnant women in Ethiopia, although the prevalence of intestinal parasites was slightly higher 24.7% by FEC [20]. Another study which compared the FEC technique with other methods reported a prevalence of 39.2% [24]. Many other studies have reported likewise [8, 14]. The

relatively lower prevalence in the present study could perhaps be attributed to the implementation of de worming as recommended by the WHO^[34, 35]. However other studies have also reported high prevalence of intestinal parasites, contrary to findings from the current study. One of such studies recorded a prevalence of 63.3%^[13] and another which evaluated different concentration techniques for stool evaluation reported a prevalence of 65.57%^[23]. Likewise another study among school age children in Ethiopia reported a prevalence of 74.6% for intestinal parasites^[26].

These discrepancies might be due to differences in the age groups of the study participants. Children of school age are prone to infections with intestinal parasites, especially those transmitted fecal-orally. This may be responsible for the high prevalence of intestinal parasites among school age children in Ethiopia^[26]. The present study was however carried among participants of all ages with only 13.21% (28/212) being within the school age (≤ 19 years) group. Furthermore up to about 49.06% (104/212) of the study participants were within the age group of 20-30 years, and this age group also had the highest percentage (44.44%; 16/36) of those infected. Most (62.26%; 132/212) of the study participants were females and they also constituted the majority (77.78%; 28/36) of those with intestinal parasites. The females might have been more exposed because they are closer to the children who are the most vulnerable to especially infections with fecal-oral transmission routes.

The effectiveness of the wet mount technique compared to the FEC technique is shown on table 2. The prevalence of intestinal parasites by wet mount and FEC techniques were 06.60% (14/212) and 16.98% (36/212) respectively. The difference between the two techniques was significant [P = 0.0424] and generally the kappa value ($k=0.3889$) of the test results between both techniques is poor. This means that there is a significantly poor agreement between the results of the wet mount and

FEC techniques. Specifically for the diagnosis of *G. lamblia*, there was a perfect agreement ($k=1$) between both techniques. The kappa value ($k = 0.75$) for the diagnosis of *E. coli* was also good. However the kappa value ($k = 0.375$) for the diagnosis of *E. histolytica* was poor. And worse still was the kappa value ($k=0$) for the diagnosis of *I. bustchli*, *F. hepatica*, *E. vermicularis* and hookworm. There were zero agreement in the test results of the wet mount and FEC techniques, for the diagnosis of these parasites.

Similar results were earlier gotten in another study, the prevalence of intestinal parasites by FEC was 24.7% as compared to 18.80% by direct wet mount^[20]. The kappa value however varied from what was gotten in the current study. The kappa value for the detection of hookworm in that study was 0.81^[20], contrary to that of the current study which was 0. These differences might have been due to differences in the worm burden of the patients. The parasite load was generally low for all the intestinal parasites in the reported fact that FEC technique was more sensitive than the direct wet mount technique^[13, 23, 24]. The FEC technique had equally been proven to be more preferable than the kato-katz techniques^[26, 28, 29]. In line with the current study, Pakdadet *al.*, 2018 reported that the kappa agreement [between the direct wet mount and FEC techniques] for the diagnosis of helminthic infections was lower than for the diagnosis of protozoans. In the present study the detection of *G. lamblia* by both techniques had a perfect k agreement ($k=1$), followed by the detection of *E. coli* ($k=0.75$) and *E. histolytica* ($k=0.375$), these are all protozoans. However for the detection of all the helminthes (*F. hepatica*, *E. vermicularis* and hookworm), the K value was zero. This confirms the fact that the kappa agreement between the direct wet mount and FEC techniques are better for protozoan infections than for helminthic infections.

The specificity of the direct wet mount technique which reflects the probability that the test will be

negative amid the truly negative cases, was 100% for all the parasites detected. This means that among all those who tested negative by the FEC technique, none had tested positive by the direct wet mount technique. Likewise the positive predictive value (PPV) which is the likelihood that the disease is present when the direct wet mount test is positive. The PPV of the wet mount technique in the diagnosis of each parasite was a 100%. This means that all the cases which tested positive with the direct wet mount technique also tested positive with the FEC technique. However the sensitivity which reveals the prospects that the direct wet mount test will be positive among those who are diseased, varied according to the parasite species. This shows that some of the study participants with the disease actually tested negative by the direct wet mount, but positive by the FEC technique. Likewise the negative predictive value (NPV) which is the chance that the disease is not present when the test is negative. The NPV also varied according to the parasite species. In some cases the direct wet mount test actually reported negative when by the FEC technique the disease was present.

The sensitivity of the wet mount technique in diagnosing helminthic infections was generally poor. However the sensitivities were generally good in the diagnosis of protozoan infections (except *I. bustchli*). The sensitivities for the diagnosis of *G. lamblia*, *E. coli*, and *E. histolytica* were 100%, 80% and 61.54% respectively. But for the diagnosis of *F. hepatica*, *E. vermicularis*, *I. bustchli* and hookworm, sensitivities were all 50%. This result is contrary to what was gotten from a previous study. The sensitivity of direct wet mount technique was 3% while that of FEC was 50%^[8]. These techniques were compared alongside the kato-katz and the Spontaneous sedimentation techniques, using their combined results as “gold” standard. These discrepancies might be due to the fact that the direct wet mount and FEC methods were compared with more sensitive techniques. In another study which evaluated the wet mount, FEC and kato-katz techniques, the combined results was also used

as “gold” standard^[27]. The sensitivities of wet mounts and FEC were 38.4% and 57.1% respectively^[27]. These sensitivities are lower than that of the current study. In the current study the FEC technique was used as the “gold” standard, which explains why the sensitivities of the wet mount technique was higher. Moreover due to the cost and inaccessibility of the more sensitive techniques like the kato-katz and FLOTAC, the FEC and direct wet mount techniques were considered more convenient and accessible for use in the current study area.

CONCLUSION:

The prevalence of intestinal parasites in the current study is low and most common among young adults (20-30year) and females who also constitutes the majority of the study participants. The prevalence of intestinal parasites by the direct wet mount technique was significantly lower than what was detected by the FEC technique. Although the specific sensitivities and NPVs of the direct wet mount technique for the diagnosis of each intestinal parasite detected in this study were mostly less than 100%, the specificities and PPVs were all 100%. Therefore the performance of the direct wet mount technique was generally good and moderate for the diagnosis of protozoan and helminthic infections respectively. In order to ensure increased accuracy of the diagnosis of intestinal parasites in the study area, the routine direct wet mount technique should be complemented by the FEC technique.

Data Availability and Funding Statement

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