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Performance evaluation of three laboratory diagnostic methods for intestinal parasitic infections at rural Bahir Dar, Northwest Ethiopia: A cross-sectional study

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ABSTRACT

Introduction: Even if the prevalence of intestinal parasites is high in Ethiopia, we still use only direct wet mount method for laboratory diagnosis of intestinal parasitic infections, having low sensitivity, and this significantly increases false-negative results. Therefore, performance evaluation of three laboratory diagnostic methods is mandatory.

Methods: Single stool sample was collected from March 2018 to June 2018, among 211 school children, and processed using wet mount, modified Baermann (MB), and Ritchie's methods. The sensitivity and negative predictive values (NPVs) at 95% confidence interval and Kappa values were calculated in terms of the gold standard method (the combined result of altogether).

Results: The overall prevalence of intestinal parasites was 60.2%. The sensitivity and NPVs of wet mount, MB, and Ritchie's methods against the "Gold standard" test were 49.6% and 56.8%, 80.3% and 77.1%, and 67.7% and 68.8%, respectively.

Conclusions: MB showed the best, and wet mount showed least performances for the laboratory diagnosis of intestinal parasitic infections.

Keywords: Wet mount; modified Baermann; Ritchie's method; Rural Bahir Dar; ethiopia

INTRODUCTION

Intestinal parasitic infection is a condition in which the gastrointestinal tract of human is infected with parasites residing in the intestine (1). It is estimated that about 3.5 billion people in the world are infected with intestinal parasites (2). They are more prevalent among school children as compared to the general population (3,4). About 12% of the global disease burdens are observed among school children with age ranges from 5 to 14 years (4).

The most prevalent intestinal parasites that cause infection in the human gastrointestinal tract are *Giardia lamblia, Entamoeba histolytica,* and soil-transmitted helminths (STHs) *Ascaris*

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lumbricoides, Trichuris trichiura Hookworm species (Hook worm sp.), *Strongyloides stercoralis, Taenia saginata,* and *Hymenolepis* sp. (1,5).

In Ethiopia, intestinal parasitic infection is the second most predominant cause of outpatient morbidity (2,6). The most prevalent intestinal helminths are *A. lumbricoides*, followed by *T. trichiura* and Hookworm sp. (7,8). Negussu et al. (9) reported that, in Ethiopia, the number of people living with STHs in endemic areas is estimated at 79 million, comprising 9.1 million pre-school aged, 25.3 million school-aged, and 44.6 million adults. Despite this prevalence, wet mount method has been still used as a diagnostic method at all levels of health facilities in Ethiopia. Thus, evaluating additional laboratory diagnostic methods for intestinal parasitic infections that has not been used before in the country is mandatory as discussed in subsequent paragraphs below.

Wet mount method is the most commonly and routinely used method for the diagnosis of intestinal parasitic infections in Ethiopia due to its feasibility as compared to other techniques (10,11). However, it has limitations such as lack of sensitivity as it used a small amount of stool sample (11).

Modified Baermanns (MBs) method has a greater sensitivity to detect most intestinal parasites including larva of *S. stercoralis* (12). It can be used routinely in developing countries such as Ethiopia because it requires less costly materials, used in the absence of a centrifuge, and not time-consuming (13,14).

Finally, Ritchie's method is the sedimentation concentration in diagnosis of a wide range of intestinal parasites. This method is used for detecting intestinal nematode eggs that are not detected by wet mount method (15). This method is also better to concentrate intestinal helminthic eggs and intestinal protozoan cyst in fecal samples present in small numbers (2,16).

Lack of laboratory diagnostic methods having a better sensitivity and specificity for intestinal parasitic infections impairs appropriate patient management and accurate epidemiological data and thus limits the disease control measure. Hence, evaluating the performances of laboratory diagnostic methods is essential for improving the diagnostic performances of these methods.

METHODS

Study area and design

A school-based cross-sectional study was conducted on school children at Meshenti and Gedro elementary schools in rural Bahir Dar, from March 2018 to June 2018. Bahir Dar is a capital city of Amhara Region lying at an altitude of 1900 m above sea level, 1419 mm annual rainfall, and average annual temperature of 19.6°C. Based on the (17) census conducted by the Central Statistical Agency of Ethiopia, Bahir Dar special zone has a total population of 221,991, of whom 108,456 are men and 113,535 women and 81.16% are urban inhabitants.

Sample size determination and sampling technique

We used Buderer's formula to evaluate different diagnostic methods. Since there is no previous study done in the study area, 50% prevalence, 95% confidence interval (CI), 5% marginal error, and 10 % non-response rate, a total of 211 students were selected.

Finally, the sample size was proportionally allocated for each class and grade, taking the total number of students in each category into consideration. Class roster was used as a sampling frame, and a systematic random technique was employed to select study participants.

Stool sample collection and processing

All study participants were informed about the purpose of the study. About 20–22 g single stool sample was collected from each study participants using clean stool cup and processed by wet mount, MB, and Ritchie's methods. Each method was evaluated against the gold standard (the combined results of the three methods altogether based on Bayesian rule) (18).

Laboratory procedures of the three diagnostic methods

Wet mount method

In the wet mount, fresh stool samples (2 mg of stool) were put on a slide with wooden applicator, emulsified with a drop of physiological saline (0.85%) for

diarrheic and semi-solid samples. For formed stools, iodine was used. Then, covered with cover slide and examined under microscope using first ×10 objectives and then ×40 objectives.

MB method

The test was performed by 2–5 g of fresh stool sample homogenized in 10 ml of saline solution and filtered through surgical gauze into a 50 ml plastic tube, which is then filled with more saline solution, plugged, and shaken vigorously. Then, the tube is left to stand for 45 min, after which the supernatant is removed and a sample is taken from the bottom and put on a slide for microscopy (19).

Ritchie's method

For this, 0.5 g fresh stool sample was added in the sample collecting tube containing 2.5 ml of formalin and 1 ml of ethyl acetate and the sample was well mixed and then centrifuged. Finally, the supernatant was discarded, the sediment mixed and put on the microscope slide for examination (20).

Quality control

To maintain the reliability of the study findings, 15% were randomly selected and reexamined at the end by experienced laboratory technologist who was blind for the first examination result.

Data analysis

Data were analyzed using Statistical Package for the Social Sciences statistical software version 20. Since there is no gold standard method to detect intestinal parasites, the combined results of all methods altogether can be used. The sensitivity, specificity, and negative predictive values (NPVs) at 95% CI and Kappa values of each technique were calculated against the gold standard.

Ethical considerations

Ethical clearance was obtained from Bahir Dar University College of Medicine and Health Science ethical review committee before start the study. A supportive letter was obtained from Amhara Regional Health Bureau. A written informed consent was also obtained from every study participant, parent, or guardian. Those study participants who were positive for intestinal parasites were referred to the nearby health centers for treatment.

RESULTS

A total of 211 study participants were participated in the study and 60.2% were positive by the gold standard method. The prevalence of intestinal parasites using 3wet mount, MB, and Ritchie's methods was 29.9%, 48.3%, and 40.8%, respectively (Table 1).

The sensitivity, specificity, and NPVs of wet mount method for the diagnosis of intestinal parasitic infections were 49.6%, 100%, and 56.8%, respectively. Agreements of this method with the gold standard for the diagnosis of intestinal parasitic infections were moderate (k = 0.439) (Table 2).

The sensitivity, specificity, and NPVs of MB method were 80.3%, 100%, and 77.1%, respectively. Agreements of the test with the gold standard for the diagnosis of intestinal parasitic infections were very good (k = 0.77) (Table 3). The sensitivity, specificity, and NPV of Ritchie's method were 67.72%, 100%, 67.2%, respectively. Finally, agreements of the test with the gold standard for the diagnosis of intestinal parasitic infections were very good (k = 0.63) (Table 4).

DISCUSSION

In this study, the overall prevalence was 60.2%. This was lower than a study in Northwest Ethiopia,

TABLE 1. The performance of diagnostic methods for intestinal parasitic infections

Results				
Methods	Number examined	Positive *n* (%)	No ova/parasite *n* (%)	Total *n* (%)
Wet mount	211	63 (29.9)	148 (70.1)	211 (100)
MB	211	102 (48.3)	109 (51.7)	211 (100)
Ritchie's	211	86 (40.8)	125 (59.2)	211 (100)
All methods	211	127 (60.2)	84 (39.8)	211 (100)

MB: Modified Baermann

TABLE 2. The performance of wet mount method against the gold standard for the diagnosis of intestinal parasitic infections (%)

Gold standard method							
Wet mount	Positive	Negative	Total	Sensitivity (95 % CI)	Specificity (95 % CI)	NPV (95 % CI)	Kappa
Positive	63 (49.6)	0 (0)	63 (29.9)	49.6 [58.8–75.7]	100 [95.7–100]	56.7 [61.4–72.5]	0.63
Negative	64 (50.4)	84 (100)	148 (70.1)				
Total	127 (100)	84 (100)	211(100%)				

CI: Confidence interval

TABLE 3. The performance of MB against the gold standard method for the diagnosis of intestinal parasitic infections

Gold standard method							
MB	Positive (%)	Negative (%)	Total (%)	Sensitivity (95 % CI)	Specificity (95 % CI)	NPV (95 % CI)	Kappa
Positive	102 (80.3)	0 (0)	102 (48.3)				
Negative	25 (19.7)	84 (100)	109 (51.7)	80.3 [72.3–86.8]	100 [95.7–100]	53.5 [70.3–82.7]	0.77
Total	127 (100)	84 (100)	211 (100)				

CI: Confidence interval, MB: Modified Baermann

TABLE 4. The performance of Ritchie's method against the Gold standard for the diagnosis of intestinal parasitic infections

Gold standard method							
Ritchie's	Positive	Negative	Total	Sensitivity (95 % CI)	Specificity (95% CI)	NPV (95 % CI)	Kappa
Positive	86 (67.7)	0 (0)	86 (67.7)				
Negative	41 (32.3)	84 (100)	41 (32.3)	67.72 [58.8–75.7]	100 [95.7–100]	67.2 [61.4–72.5]	0.63
Total	127 (100)	84 (100)	211 (100)				

CI: Confidence interval

79.8% (21) and East Gojjam Zone 83.4% (22) and higher than 35.44% in Benishangul-Gumuz (23) and in Bahir Dar, Northwest, 59.8% (4). This difference might be due to the geographical difference, or it might be associated with a difference in parasitological methods.

In this study, wet mount method confirmed 29.9%, modified Baermann confirmed 48.3%, and Ritchie's method confirmed 40.8%. This result agrees with the previous studies (13,14).

Wet mount method exhibited very low sensitivity for the detection of other intestinal helminths. This was in agreement with a study done in Bahir Dar (4). A small amount of fecal material used in this technique might be the reason for lower detection capacity of the method. Wet mount method exhibited the lowest sensitivity of 49.6% and NPVs of 56.76% as compared to MB, with a sensitivity of 80.3% and NPVs of 77.1%, and Ritchie's method with sensitivity 67.7% and NPVs of 68.8% for the detection intestinal parasitic infections. This suggested that the use of wet mount method for the diagnosis of intestinal parasitic infections is insufficient and the use of another diagnosing method is mandatory to decrease misdiagnosis.

In the current study, 48.3% of the intestinal parasitic infections were detected by the MB method with a sensitivity of 80.3% and NPVs of 77.1%. This method is promising to use it as a routine laboratory diagnostic method for intestinal parasitic infections. Moreover, MB requires less costly materials even we can use in the absence of centrifuge. However, lack of previous similar studies made difficulty in making rigorous discussion on this finding.

In the present study, Ritchie's method detected 23.7% of Hookworm sp. followed by *A. lumbricoides* 5.7%. This is in agreement with other studies done previously (24). However, this study disagrees with a study done in Rio de Janeiro, Brazil. This high value may be due to the use of greater size of the coverslips measuring 24 mm × 32 mm to increase the spread of fecal material than those used in routine laboratory analysis (22 mm × 22 mm) (25).

Based on parasite recovery, our results confirmed that Ritchie's method (40.8%) is higher than the direct wet mount method (29.9%). This result was in agreement with other studies done previously (2,26). Furthermore, the sensitivity and NPVs of Ritchie's method were 67.7% and 67.2 % which were higher than wet mount method 49.6% and 56.8%.

CONCLUSIONS

In this study, MB showed the best performance as compared to wet mount and Ritchie's methods, and Ritchie's method showed better performance as compared to wet mount method. Therefore, it is preferable to use MB, in complement with Ritchie's and wet mount methods as a routine laboratory diagnosis of intestinal parasitic infections.

Limitation of the study

The limitation of this study was that all the combined results of the three methods are highly influenced by parasite prevalence. Thus, the same method will have different values in different areas of prevalence.

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COMPETING OF INTEREST

We authors declare that we have no conflicts of interest.

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