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Evaluation of Stool Concentration Techniques in the Detection of some Parasites among Almajiri School Children in Sokoto Metropolis

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Abstract

Infection with intestinal parasites is one of the main health issues in poor nations. It is estimated to affect 3.5 billion people worldwide, and 450 million people are thought to be ill as a result of such illnesses, with children making up the majority of those who are ill. By using several stool concentration techniques, this study was aimed at identifying parasite ova in the stool of Almajiri school children in Sokoto. The Formol-ether concentration technique, which is regarded as the gold-standard, Sheather's sugar floating technique, and saline floatation technique were employed to detect parasite ova in stool samples taken from Almajiri school children. A total of 137 stool samples were collected from Almajiri school children and each sample was examined by these concentration techniques. A prevalence of 37.9% of intestinal helminthiasis was observed by Formol-ether concentration technique. The sensitivity and specificity of Formol-Ether concentration technique were 96.2% and 97.8% respectively, followed by Sheather's sugar floating technique 88.5% and 93.8% and the lowest sensitivity and specificity of 76.9% and 90% respectively were obtained in the saline floatation technique. There was a high prevalence of intestinal helminthiasis among Al-majiri school children, sheather's sugar floatation technique was as sensitive as the Formol-ether concentration technique and more sensitive than the Saline floatation technique. Therefore, Sheather's sugar floatation technique is recommended as a better alternative in the diagnosis of intestinal helminthiasis.

Keywords: Almajiri school, Concentration, Techniques, Sokoto, Stool.

INTRODUCTION

There are more than 60 different types of nematodes that can infect people. More than 1 billion people are estimated to be infected with *Ascaris lumbricoides*, hookworms (*Ancylostoma duodenale* and *Necator americanus*), and *Trichuris trichiura* (Tille, 2014). According to Jamala *et al.* (2018), the global burden of soil-transmitted helminthiasis is estimated to be 39 million Disability Adjusted Live Years (DALYs). Intestinal parasite prevalence is typically high in sub-Saharan Africa, and whereas the frequency of Intestinal parasitic infections (IPIs) is 50% in affluent countries, it can reach 95% in developing nations (Tegegne *et al.*, 2018). Intestinal parasites, such as schistosomiasis and soil-transmitted helminthiasis, have been shown to negatively impact children's physical and mental development (Gashaw *et al.*, 2015). The immediate effects of soil-transmitted helminth infections include diarrhea, abdominal pain, and low hemoglobin levels. However, the long-term effects of these infections are much more dangerous, as those who have them exhibit diminished cognitive abilities, intellectual capacity, and lower work productivity (Salam and Azam, 2017). According to the CDC (2013), there are between 807 million and 1.121 billion people worldwide who have *Ascaris lumbricoides*, 604 million to 795 million who have whipworm (*Trichuris trichiura*), and between 57 million and 740 million who have hookworm (*Ancylostoma duodenale* and *Necator americanus*). Helminthiasis prevalence is linked to insufficient hygienic conditions and poverty. According to Jamala *et al.* (2018), helminthiasis affects about one-third of the global population, primarily rural people.

Intestinal parasitic infections (IPIs) continue to be the most important and unresolved public health issue in underdeveloped nations like Ethiopia because of a lack of experience in health promotion, a precarious economic situation, and a low level of environmental cleanliness (Tegegne *et al.*, 2018). Albendazole or Mebendazole can treat the illness, but eradicating it is difficult due to the potential of re-infection among residents of the affected areas due to the faeco-oral and penetration-via-skin transmission patterns of soil-transmitted helminths (Salam and Azam, 2017). The typical method for identifying helminths that are spread through soil is to look for eggs in a stool sample under a microscope (CDC, 2017). It does, however, have some limitations, such as a lack of sensitivity, especially when the diagnosis is based only on a microscopic examination of stool samples. This is probably due to irregular or intermittent parasite shedding or the small amounts of parasites discharged in feces (Mergani *et al.*, 2014). Children in preschool and school-aged (including adolescent) groups usually have the highest numbers of intestinal worms and schistosomes for unidentified reasons. Their scholastic performance is impacted by their stunted growth, lower physical fitness, and deficiencies in memory and cognition (Hedley *et al.*, 2015). Concentration methods improve the sensitivity of copromicroscopic techniques, increasing the possibility of discovering parasite organisms (Acharya, 2012). Although there are alternative methods of concentration, the sedimentation and flotation processes are the most used. Concentration techniques have been utilized in clinical laboratories ever since Ridley established their effectiveness in 1948 (Manser *et al.*, 2016). Giving the enormous effect of the intestinal parasitic infections and need to rapidly detect and treat these infections, this study aimed at the detection of some parasites among Almajiri school children in Sokoto metropolis by comparing three stool Concentration techniques.

MATERIALS AND METHODS

The research was done in some selected Almajiri schools. The sample size used was obtained using the formula by Ibrahim (1997) and a prevalence rate of 9.1% according to studies by Enimien *et al.* (2014) as indicated below:

$n = Z^2 P Q / d^2$

Where:

n = Minimum sample size

d = desired level of significance (0.05)

z = Confidence interval (1.96)

p = Prevalence rate (9.1%)

q=1-P= (1-0.091) = 0.909

Using this formula, the minimum number of samples was;

$n=1.96^2 \times 0.091 \times 0.909 / 0.05^2 = 127.12 = 127 \approx 130$

10% attrition rate (13) was added to the sample size: 130+13=143 samples was the calculated however 137 sample was collected and used for the analysis.

Inclusion and Exclusion Criteria

All uncontaminated stool samples (formed, semi-formed and watery) obtained from consented subjects were used for the study. Stool contaminated with urine or mixed with soil and those from unconsented subjects were excluded from the study.

Informed Consent

The informed consent was read to the understanding of the study subjects in the language they understand before they were enrolled for the study.

Ethical Consideration

Approvals for this study was obtained from Ministry of health (reference number: SMH/1580/V IV) and verbal consent sought from the head of the schools in order to have access to the children and their samples (stool).

Laboratory Procedure

Formol ether concentration technique was used as described by Cheesbrough (2009). About 1g of pea-sized feces were emulsified in 4ml of 10% formal saline in a screw-capped tube or container. The bottle was sealed, a tiny amount of 10% formal saline was added, and everything was properly combined by shaking. After being sieved, the emulsified feces' sieved suspension was collected in a beaker. Following that, the suspension in the conical flask received 3ml of diethyl-ether. The tube was then sealed and mixed for one minute. After slightly releasing the stopper, the tube was centrifuged for one minute at 3000 rpm. The coating of excrement on the tube's side was broken open using the stem of a plastic bulb pipette. The tube was inverted upside down to remove the diethyl-ether. A plastic bulb pipette's stem was used to pry open the layer of faeces on the tube's side. The diethyl ether, feces, and formaldehyde were then removed from the tube, leaving only the sediment. After that, the tube was put back in its upright position so that the liquid coming from the side could drain to the bottom. The sediment was then re-suspended and mixed by tapping the tube. The sediment was then placed to a glass slide, covered with a cover slip, and examined with a 40x objective to detect the helminths while the condenser iris was kept sufficiently closed to provide good contrast.

Sheather's sugar floatation method

Sheather's sugar floatation method was carried out according to Wichit *et al*. (2015). In a cup, 10 ml of sugar flotation solution and 2 grams of faeces were well combined. The mixture was then filtered through two layers of gauze into a conical 15 ml centrifuge tube. The tongue depressor was used to extract the liquid from the faeces that was still in the gauze strainer. The tube was removed, put on a test-tube rack, and fully filled with sugar flotation solution following a 5-minute 1,000 x g centrifugation. The tube was covered with a 22 × 22 mm coverslip, which was then removed after 10 minutes and put on a glass slide. The coverslip as a whole was then examined under a light microscope.

Simple salt floatation method

As stated by Parameshwarappa *et al*. (2012), a straightforward salt flotation approach was used. In a 20 ml conical glass test tube, 1g of human waste was briefly emulsified with 4 ml of saturated salt solution. The mixture was then thoroughly swirled before more salt solution was added and maintained swirling until the container was almost filled. A glass slide was placed on top of the tube, covering the portion that was in touch with the fluid, after any coarse debris that floated up was cleared away. The tube was then set on a level surface. After then, it was permitted to stand for 30 minutes. The slide was then removed, and any eggs or cysts were checked for.

The sensitivity and specificity of the three different concentration methods was determined accordingly as described by Baratloo *et al*. (2015).

RESULTS

Out of the 137 samples collected from four (4) Al-Majiri schools in Sokoto metropolis, the prevalence of intestinal helminthes was 37.9%. Parasites detected include *Ascaris lumbricoides*, *Trichuris trichiura* and Hookworm (Table 1). The highest prevalence of 71.4% was detected from Al-Majiri School Children within the age group of 4-6 years followed by 38.5% in the age group of 13-18 years (Table 1). The prevalence of helminthes in Al-majiri School Children in the age group of 7-9 was 29.2% while the lowest prevalence of 28.8% was observed among 10-12 years Almajiri School Children (Table 1).

The distribution of parasites detected in Almajiris with respect to risk factors is presented in Table 2. The highest prevalence 42.7% was observed in children that use well water for drinking purpose followed by Children using stream/river water as drinking source that had a prevalence of 40%, no parasite was detected among the children whose drinking water is Tap (Table 2). Almajiri children, who don't wash their hands before eating, showed a higher prevalence of intestinal helminthes 70.5%, those who wash their hands before eating showed a lower prevalence of helminthes 22.6%. Almajiri School Children Walking bare-footed had a prevalence of 72.2%, and those not walking bare-footed had a lower prevalence 25.7% of helminthes (Table 2). The prevalence of intestinal helminthes among Almajiri Children using pit latrine was 40.5% in contrast to children that use open space for defecation that had a prevalence of 27% (Table 2).

The Distribution of Parasites based on concentration methods was shown in Table 3. A total of 137 samples were screened using three concentration methods. Formol-ether detected 50 positive samples with 66 ova present, Sheather's sugar detected 46 positive samples with 58 ova present and Saline floatation detected 40 samples with 47 ova present (Table 3).

Table 4 shows types and frequency of parasites detected based on concentration methods. The formol-ether concentration technique detected a total of 66 (38.6%), Sheather's sugar floatation technique detected 58 (33.9%) and Saline floatation technique detected 47 (27.5%) (Table 4).

Table 5: shows sensitivity and specificity of the three concentration methods used in the detection of parasites. Among the methods, Formol-ether concentration technique had the highest sensitivity 96.2%, followed by Sheather's sugar floatation technique 88.5% and the least sensitive was Saline floatation technique with a sensitivity of 76.9% (Table 5). The specificity of the concentration methods was as follows; formol-ether had the highest (97.8%) followed by Sheather's sugar with 93.8% and the least specificity was 90.0% observed in the saline floatation method.

Table 1: Distribution of parasites detected in Almajiri children with respect to age

Age group	No of samples screened	Detection Frequency	Percentage (%)
4 -6	14	10	71.4
7-9	24	7	29.2
10-12	40	13	32.5
13-18	59	22	37.3
TOTAL	137	52	52/137=37.96

Table 2: Distribution of parasites with respect to risk factors

Studied Risk factors	**No of samples screened**	**Detection frequency**	**Prevalence (%)**
Water source			
Tap water	15	0	0
Well water	117	50	42.7
River/stream water	5	2	40.0
Hand washing			
Yes	93	21	22.6
No	44	31	70.5
Type of Toilet			
Pit latrine	37	15	40.5
Open space	100	27	27.0
Walking barefooted			
Yes	36	26	72.2
No	101	26	25.7

Table 3: Distribution of Parasites based on concentration methods

Concentration method	No of samples screened	No of positive samples	Percentage of positive samples (%)	No of Ova detected	Percentage of ova detected (%)
Formol-ether	137	50	36.5	66	38.6
Sheather's sugar	137	46	33.6	58	33.9
Saline	137	40	29.2	47	27.5

Table 4: Distribution of intestinal helminthes based on concentration methods

Concentration method	No of samples examined	Parasite type			No and percentage prevalence (%)
		Hookworm	*A. lumbricoides*	*T. trichiura*	
Formol-ether	137	35	27	4	66 (38.6)
Sheather's sugar	137	34	22	2	58 (33.9)
Saline floatation	137	31	16	0	47 (27.5)

Table 5: Sensitivity and Specificity of Concentration methods used in the detection of parasites

Concentration methods	No of samples examined	No of parasites detected	Sensitivity (%)	Specificity (%)
Formol ether	137	66	96.2	97.8
Sheather'ssugar Floatation	137	58	88.5	93.8
Saline floatation	137	47	76.9	90.0

DISCUSSION

One of the most significant causes of morbidity and mortality worldwide is intestinal parasite infections. The most prevalent intestinal parasites in this study were hookworm, *A. lumbricoides*, and *T. trichiura*, with prevalence rates of (37.9%), which were higher than the findings by Mohammed *et al.* (2015) in Kaduna, Nigeria (18.61%), but lower than the results from Jigawa (Yahaya *et al.*, 2015), (54.8%), and Imo state (Odinaka *et al.*, 2015), (30.3%). These findings are not surprising given that Tegegne *et al.* (2018) explained that intestinal helminthic infections are frequent in low-income and

socioeconomically disadvantaged areas in the tropics and subtropics, where poverty, overpopulation, subpar environmental sanitation, and a lack of education are more pronounced.

In this study, the age group 4-6 years was the most affected with the highest prevalence in the study, while the age group 7-9 years had the lowest prevalence. Higher infection rate in the 4-6 years age group may be due to the fact they are not well taken care of in terms of food and feeding habits, in the same vein, they were young and immature hence are less concerned about their health and well-being. This was in contrast with the findings by Iduh *et al*. (2015) who observed that 1-15 years aged group and 6-10years had the highest prevalence of 36.8% and 21.1% respectively.

In the use of toilet, subjects that use pit latrine had a higher prevalence (15/37, 40.5%), while those that use open space had a lower prevalence (27/100, 27.0%). This could be explained by the fact that poor personal hygiene and usage of faecal-contaminated floor may contribute to high level transmission of intestinal parasitic infection. These observations were also made by Chigozie *et al*. (2007) and Obiukwu *et al*. (2008).

Hookworm infection was found to be more prevalent 17.5% compared to *A. lumbricoides* 13.1% and *T. trichiura* 7.2% and *S. mansoni* or *Taenia* spp. was not detected in this study. This may probably be due to the fact that the study was done in a metropolis (urban centre) hence access to river was very difficult as in the case of *S. mansoni*. In the case of taeniasis, the Al-majiri hardly eats meat routinely, hence reducing their chances of having contact with this parasite. High Hookworm infection 24/137 (17.5%) was probably due to the fact that even some adults among the study group walk bare-footed, giving the parasite chance of entry following contact.

Sheather's sugar floatation technique provided almost a good detection rate when compared to the formol-ether concentration technique Rojekittikhun *et al*. (2015). The results of this also confirmed that the Sheather's sugar floatation technique is almost as good as the formol-ether concentration technique, while the saline floatation technique lagged behind. The study findings exhibited clearly that the Formol-Ether Concentration Technique had the highest sensitivity 96.2%, followed by Sheather's Sugar Floatation Technique 88.5% and the least was the Saline Floatation Technique 76.9%. As regards to specificity, the Formol-ether method had the highest 97.8%, followed by the Sheather's sugar 93.8% and the least was saline floatation 90.0%, and these observations were similar to the findings by Rojekittikhun *et al*. (2015) in Kanchanaburi Province, Thailand.

The study notes that helminthes larvae were not easily concentrated in formol-ether technique as such in the absence of materials for Formol-Ether Concentration Technique, the Sheather's Sugar Floatation Technique may be carried out knowing it is almost as sensitive as Formol-Ether Concentration Technique in the detection of intestinal helminthes.

The marked similarity between the sensitivity of the Formol-Ether Concentration Technique and Sheather's Sugar Floatation suggested the need to incorporate this technique into routine laboratory diagnostics procedure. The sugar which is the major component of the Sheather's sugar floatation solution is very cheap and easily accessible to almost every individual. The floatation solution also does not deteriorate on prolonged storage. This also applies to the saline floatation method where salt is easily accessible and centrifugation is not necessary for this concentration method.

CONCLUSION

There was a high prevalence of intestinal helminthiasis among Al-majiri. Sheather's sugar floatation technique was found to be as sensitive as the Formol-Ether Concentration Technique and more sensitive than the Saline floatation technique. Therefore, for economy and simplicity, Sheather's sugar floatation technique can be used as an alternative in the diagnosis of intestinal helminthiasis due to its relatively high sensitivity and specificity.

Limitation

This study's primary drawback is the use of a single stool sample from each study participant to determine the prevalence of parasites. The intra- and interstool variation of egg/ova was therefore unavailable Additionally, a single saline wet mount and a formol ether concentration approach for each stool sample might have impacted on the accuracy of the egg/ova count.

Competing Interests

Authors have declared that no competing interests exist.

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REFERENCES

Acharya, T. (2012). Formal-ether sedimentation technique for the concentration of stool parasites and Laboratory diagnosis of parasitic disease. Retrieved on 2nd March,2018, From http://.microbeonline.com/formol-ether-sedimentation-techniques/.

Baratloo, A., Hosseini, M., Ahmed Negida, A. and El Ashal, G. (2015). Simple Definition and Calculation of Accuracy, Sensitivity and Specificity, Emergency 3(2): 48-49.

Centers for Disease Control and Prevention (CDC). (2013). Soil-transmitted helminths: Global health division of parasitic diseases. Available at www.cdc.gov/parasites/sth/.Retrieved on 3rd March, 2018.

Centers for Disease Control and Prevention (CDC). (2017). Helminths, Soil-Transmitted: Infectious diseases related to travel. Available at www.cdc.gov/parasites/sth/.Retrieved on 3rd March, 2018.

Cheesbrough, M. (2009). District laboratory practice in tropical countries(part-1) (2ndedn). England: Cambridge University Press, pp. 209-235

Chigozie, J., Kelvin, O., E., Patrick, G., O., Nelsonm, C., A. and Emmanuel, A., (2007). Soil transmitted Helminth infection in school children in Eastern Nigeria; the public health implication. International Journal of third world medicine. (4)1. [Crossref]

Enimien O. J., Fana S. A. and Emmanuel W. B., (2014). Intestinal helminthic infection in Numan (Northeast Nigeria). International Journal of Innovation and Applied Studies5(2): 102-105.

Gashaw F., Aemero M., Legesse M., Petros B., Teklehaimanot T., Medhin G., Berhe N., Mekonnen Y. and Erko B. (2015). Prevalence of Intestinal Helminth infection among school children In Maksegnit and Enfranz Towns, Northwestern Ethiopia, with Emphasis on Schistosoma mansoni Infection. Parasites and Vectors.8(567): 1-8. [Crossref]

Hedley, L., Robert, L. and Wani, S. (2015). Helminth infections: diagnosis and treatment. The pharmaceutical journal.295(962): 1-3.

Ibrahim, T. (1997) Sample size determination In Research Methodology and Dissertation writing for health and allied health professionals (1stEdn). Nigeria: Lucas, A.O, pp. 74-75.

Iduh, M. U Mohammed K., Isah I., Ashcroft O. F., Garba M. K and Nataala, S. U (2021). Gastro Intestinal Helminths among Hausa-Fulani inWamakko and Tambuwal Local Government Area of Sokoto, Nigeria. International Journal of Pathogen Research, 7(4): 22-37;[Crossref]

Jamala U.L., Eze N.C. and Nduka F.O. (2018). Prevalence and intensity of intestinal helminth infections and associated risk factors among school-aged children in Abua/Odual Local Government Area, River state. Journal of Applied Life Sciences International.16(2): 1-7. [Crossref]

Manser, M.M., Saez, A.C.S. and Chiodini, P.L. (2016). Faecal parasitology: concentration methodology needs to be better standardized. Neglected Tropical Diseases10(4): 1-16. [Crossref]

Mergani H.M., Mohammed A.S., Nawed K., Meraj B. and Khan A. (2014). Detection of Intestinal Protozoa by using Different methods.Journal of dentistry and medical research; 2(2): 28-32. [Crossref]

Mohammed, M., Vantsawa P.A., Abdullahi, U.Y. and Muktar, M.D. (2015). Nutritional Status and Prevalence of Intestinal Schistosomiasis among Al-majiriPopulation in Kawo District of Kaduna Metropolis, Kaduna State-Nigeria.Journal of Bacteriology and Parasitology, 6(4).[Crossref].

Obiukwu, M., O.,Umeanaeto, P., U.,Eneanya, C., I. and Nwaorgu, G., O. (2008), Prevalence of gastrointestinal helminths in school children in Mbaukwu, Anambra State, Nigeria.Nigeria journal of parasitology, 29(1), pp 15-19. [Crossref]

Odinaka, K.K., Nwolisa, E.C., Mbanefo, F., Iheakaram,A.C. and Okolo, S. (2015). "Prevalence and Pattern of Soil-Transmitted Helminthic Infection among Primary School Children in a Rural Community in Imo State, Nigeria", Journal of Tropical Medicine, vol. 4[Crossref]

Parameshwarappa, K.D., Chandrakanth C. and Sunil, B. (2012). The prevalence of intestinal parasitic infestations and the evaluation of different concentration techniques of stool examination.Journal of clinical and diagnostic research.6(7): 1188-1191.

Rojekittikhun, W., Mahittikorn, A., Prummongkol, S., Puangsa-art S., Chaisiri, K. and Kusolsuk T. (2015). Evaluation of Sugar Flotation and Formalin-Ether Concentration Techniques in the Examination of GI Parasites of Refuge Dogs and Cats in Kanchanaburi Province, Thailand. Journal Of Tropical Medicine and Parasitology, 38(1): 17-24.

Salam N. and Azam S. (2017). Prevalence and distribution of soil-transmitted helminth infections in India: Journal of Public Health. 17(201): 1-12. [Crossref]

Tegegne Y., Wondmagegn T., Worku L. and Zeleke A.J. (2018). Prevalence of Intestinal

Parasites and Associated Factors among Pulmonary Tuberculosis Suspected Patients Attending University of Gondar, Northwest Ethiopia: Journal of Parasitology Research 2018(9372145): 1-6. **[Crossref]**

Tille, P.M. and Forbes, B.A. (2014). Bailey and Scott's Diagnostic Microbiology(13thedn) St. Louis, Missouri: Elsevier, pp. 656-663.

Wichit, R., Aongart, M., Samrerng, P., Supalarp, P., Kittipong, C. and Teera, K. (2015). Evaluation of Sugar Flotation and Formalin-Ether Concentration Techniques in the Examination of GI Parasites of Refuge Dogs and Cats in Kanchanaburi Province, Thailand.Journal of Tropical Medical Parasitology, 38: 17-24.

Yahaya, A., Yakubu, B., T. and Aisha, I. (2015). Prevalence of intestinal Parasitic Helminths from Finger nails of "Almajiris" in Birnin Kudu local Government Area, Jigawa state, Nigeria. International Journal of Tropical Disease & Health, 8(2): 66-74**[Crossref]**