



## Evaluation of wet mount, formal ether, and special staining methods for detection of intestinal protozoa among food handlers in Khartoum, Sudan

Sara Abd Eljalil Zeenelabdeen Abdelkader<sup>1</sup>, Sannaa M A Osman<sup>2</sup>, Samah Awad Abdu Rahim<sup>3\*</sup>

<sup>1</sup> Department of Parasitology, Faculty of Medical Laboratory Sciences, Sudan International University, Khartoum, Sudan

<sup>2</sup> Department of Microbiology and Immunology, Faculty of Medical Laboratory Sciences, Al-zaiem Al-Azhari University, Khartoum North, Sudan

<sup>3</sup> College of Medicine, Alrayan Colleges, Madinah, Saudi Arabia

### Abstract

Undetected intestinal parasites by routine fecal examination may act as source for transmission of infection through food and drinks. Different techniques are used for detection of intestinal protozoa including direct microscopic examination, concentration techniques and fixed stained smear, however, Microscopical examination is the basic method for routine diagnosis of parasitic infection in stool specimens. 71 stool samples were collected from food handlers attending to the Public Health Laboratory, and Bashair hospital, Khartoum, Sudan. All specimens were examined microscopically for parasites using wet mount preparation, formal ether concentration technique, modified ZN-stain, and Gomori's trichrome stain. (39.4%) of food handlers were harboring commensals, or pathogens. The parasites detected were as follows: *Giardia lamblia* 6 (8.5%), *Entamoeba Histolytica* 4 (5.7%), *Entamoeba Coli* 14 (19.7%), *Iodamoeba butschlii* 1 (1.4%), *Hymenolepis nana* 3 (4.2%) and *Cryptosporidium Parvum* 1 (1.4%). The total number of parasites detected by trichrome stain was 22, by formal ether concentration technique were 21, by wet mount preparation were 18, and by modified ZN-stain was 1 parasite. Trichrome stain is more sensitive and specific than the other methods and is recommended to be added to the diagnostic procedures in the diagnosis of intestinal parasitic diseases.

**Keywords:** food handlers, intestinal protozoa, gomori's trichrome stain, wet mount, formal ether

### Introduction

Protozoa are single-celled eukaryotes that live as intracellular parasites in a variety of cells or as extra cellular parasites in the blood, intestine, or urogenital system. Protozoa can produce a sexually, which is one reason for their pathogenicity: very large numbers can produce, particularly when host defense mechanisms are impaired (Steven, 2003) [25]. Current laboratory methods for the diagnosis of cryptosporidiosis rely on either the direct examination of heat-fixed, faecal smears, or on the concentration of faecal samples and subsequent microscopic examination. A variety of staining procedures are employed to aid visualization of oocysts. The more usual ones include acid-fast stains such as carol fuchsin, Ziehl-Neelson and auramin-rhodamine stain (Webster, 1993). The prevalence of intestinal parasitic infection among food handler varies from area to area it was 29.4 in Khartoum, Sudan (Babiker et al, 2009) [5], 29.3 in northeastern India (Ghosh et al, 2014) [17], 34.9% in southern Iran (Mehrgan, 2018) [21], 52.1% in Ethiopia, (Legesse, 2019) [19] and 83.9% in Indonesia (Suriptiastuti, 2011) [26]. *Giardia lamblia*, *Entamoeba histolytica*, *Entamoeba coli*, *Cryptosporidium hominis*, *Cyclospora cayetanensis* are the most common intestinal protozoa detected in Sudan. Among food handlers, *Entamoeba coli* was most frequently detected, followed by *Giardia lamblia* and *Entamoeba histolytica* (Babiker et al, 2009) [5]. Some of these protozoa may lead to serious complication if not treated, therefore screening of food handler and treatment of infected is the key to prevent transmission of these infection. Contaminated food and drinks are the principal sources of infection for the transmissions of many diseases. Unfortunately, the practice

of purchasing food and drinking from hazardous sources exposes the population to infection. Undetected intestinal parasites by routine fecal examination may act as source for transmission of infection through food and drinks. Different techniques are used for detection of intestinal protozoa including direct microscopic examination, concentration techniques and fixed stained smear, however, Microscopical examination is the basic method for routine diagnosis of parasitic infection in stool specimens (Francis s, 2003) [27]. Previous studies compare direct microscopic examination and concentration techniques (Moges et al, 2010.; Bigwan et al, 2013.; Endris et al, 2013.; Hailu et al, 2015) [22, 7, 14, 18], however, to our knowledge there is no published data that compare between wet mount, formal ether, trichrome stain and modified ZN-stain, therefore the objective of this study to compare between these four techniques for detection of intestinal protozoa, and determine the prevalence of intestinal protozoa among food handlers in Mayo, Sudan.

### Materials and Methods

#### 1. Study Area

This cross-sectional study was conducted in the Faculty of Medical Laboratory Science Department of Medical Parasitology, University of Khartoum. The specimens were obtained from the Public Health Laboratory, Bashair hospital, ministry of health.

#### 2. Study populations

A total of 71 stools samples obtained from food handlers attended the public health laboratory, Bashair hospital for check up every 6 months, 10 were bloody diarrhea, and one was diarrhea only.

### 3. Direct smear examination

Mixing a small portion of stool taken with an applicator stick with a drop of normal saline on a slide and covered with cover slip and examined systematically under the microscope using (×10) and high magnification (×40) for observation of more details (Cheesbrough, 1987)<sup>[9]</sup>. Formal ether concentration technique, modified Ziehl-Neelsen "ZN" method for Cryptosporidium, Preservation techniques, and Trichrome stained smear were done in accordance with the previous literature (Markell et al, 1999.; Cheesbrough, 1999.; Garcai, 2001.; Eisa, 1985)<sup>[20, 8, 16]</sup>.

### 4. Data analysis

the result was analyzed using Statistical Package for Social Science (SPSS) computer program. For comparison Chi-square test was used.

### Results and Discussion

Intestinal parasitic infections cause serious public health problems, especially in developing countries with Prevalence of 30-60 % (Saab et al, 2004)<sup>[23]</sup>. Fecal oral route transmission of intestinal parasites constitutes important means of transmission through contaminated food, water, or hands (Zaglool et al, 2011)<sup>[31]</sup>. In developing countries, the rate of death by food borne disease is 2 million per year and up to 30% of the population in developed countries suffers from food borne diseases according to WHO (World Health Organization, 2007)<sup>[30]</sup>. Since parasitic infection may be chronic or asymptomatic, food handlers represent a common and persistent source of infection worldwide (Andargie, et al, 2008).; Zain et al, 2002)<sup>[4, 32]</sup>. Poor hygienic practice for food handlers exposes them to infection by different enteropathogens, leading to transmission of infection to community by contaminated food (Takalkar et al, 2010)<sup>[28]</sup>. In our study, out of 71 food handlers stool specimens, 10 of them were bloody diarrhoea and one sample of them was showed diarrhea only. Wet mount preparation detected a smaller number of positives for intestinal protozoa than formal ether concentration technique and permanent staining methods. Trichrome stain method showed the highest number of positives for intestinal protozoa that was in the preservatives stool sample (table1)

**Table 1:** Number of infected and non-infected people by each method

| ZN-stain | Trichrome stain | Formal ether | Wet mount | Infection    |
|----------|-----------------|--------------|-----------|--------------|
| 1        | 22              | 21           | 18        | Infected     |
| 70       | 49              | 50           | 53        | Non-infected |
| 71       | 71              | 71           | 71        | Total        |

**Table 2:** comparison between wet mount method and formal ether concentration technique.

| Total | Formal ether |      |       |
|-------|--------------|------|-------|
|       | + ve         | - ve |       |
| 52    | 5            | 47   | - ve  |
| 19    | 16           | 3    | + ve  |
| 71    | 21           | 50   | Total |

5 stool samples were examined microscopically by wet mount preparation were showed no parasites detection while the same 5 stool samples when were examined microscopically by formal ether concentration technique were showed the detection of *Entamoeba coli*, (*2*) *Giardia*

*Lambli*a cyst (1), (*1*) *Entamoeba butschlii* cyst (1), (*1*) *Entamoeba histolytica* trophozoite (1), this explain the importance of using formal ether concentration technique for the detection of light infection as well as mixed infections and follow up of the parasitic infection when the number of the parasites were few and very difficult to be detected by wet mount preparation. Both methods were agreed in the detection of intestinal protozoa in 16 stool samples, while three cases of stool samples showed the detection of intestinal parasites namely *Giardia*, *Lambli*a cyst, *Hymenolepis nana* ova, and *Entamoeba histolytica* trophozoite by wet preparation but were showed no parasites detected by formal ether concentration technique. In this result we showed the importance of wet preparation for the detection of *Entamoeba histolytica* trophozoite motility from fresh stool samples. The failure of formal ether concentration technique for defection of intestinal protozoa may be related to the chemical constituents. The sensitivity of wet mount preparation as compared to formal ether concentration technique was found to be 84.2%, and the sensitivity of formal ether concentration technique as compared to wet mount preparation was found to be 76.1%. In addition, there is highly significant correlation between wet mount preparation and formal ether concentration technique (p = 0.000). Our findings showed that formal ether concentration technique more sensitive than wet mount preparation. Similar findings had been reported in Gedarif state (Ali El fedl, 2004), Gezira state (Eisa, 2005), Khartoum state in 2001 (Babiker, 2001)<sup>[6]</sup>, and Eastern Sudan (Albadri, 1998).

**Table 3:** Comparison between wet mount preparation and trichrome stain technique.

| Total | Trichrome |      |       |
|-------|-----------|------|-------|
|       | + ve      | - ve |       |
| 19    | 13        | 6    | + ve  |
| 71    | 22        | 49   | Total |

43 stool samples were examined by wet mount preparation were showed no parasites detection while the same 9 stool samples when were examined by trichrome stain were showed the detection of *Entamoeba coli* cyst (5), (*1*) *Giardia*, *Lambli*a cyst (1), (*1*) *Entamoeba histolytica* trophozoite (2), (*1*) *Entamoeba coli* trophozoite (1) that it is explain the importance of using trichrome stain for the detection active infections and follow up of the parasitic infection when the number of parasites were few or very difficult to be defected by wet mount preparation also Trichrome method it was able to produce an excellent contrast between nucleus, ingested RBCs and inclusions characteristics Both methods were agreed in the detection of intestinal protozoa in 13 stool samples. While 6 cases of stool samples showed the detection of *Giardia*, *Lambli*a cyst, *Hymenolepis nana* ova (3), (*1*) *Entamoeba coli* cyst (1), (*1*) *Entamoeba histolytica* trophozoite (1), by wet mount preparation but were showed no parasites detected by trichrome stain. These results also showed the importance of wet mount preparation for the detection of *Entamoeba histolytica* trophozoite motility from fresh stool samples.

The sensitivity of wet preparation as compared to the trichrome stain was found to be 68,4%, and sensitivity of trichrome stain as compared to wet preparation was found to be 59.09%. Ten samples of bloody diarrhoea (delayed) were examined by wet mount and were showed no *Entamoeba histolytica* trophozoite or cyst detection. From 10 samples, 4

samples showed positive morphological features of *Entamoeba histolytica* trophozoite when examined by trichrome stain. These results reflect the facts that trichrome stain is a good method and could be as complementary method to wet mount method for diagnosis of amoebic dysentery.

In addition, there is a highly significant correlation between wet mount preparation and trichrome stain.

**Table 4:** Comparison between wet mount preparation and modified ZN-stain technique.

| Total | ZN- stain |      |       |                 |
|-------|-----------|------|-------|-----------------|
|       | + ve      | - ve |       |                 |
| 52    | 1         | 51   | - ve  | Wet preparation |
| 19    | 0         | 19   | + ve  |                 |
| 71    | 1         | 70   | Total |                 |

One stool sample was examined microscopically by wet preparation was showed no parasite detection, while the same stool sample when was examined microscopically by modified ZN-stain was showed the detection of *Cryptosporidium parvum* oocysts. This explain the importance of using modified ZN-stain for the detection of oocyst of *Cryptosporidium parvum* in diarrhoeal stool sample and failure of wet mount preparation due to oocyst it is transparent and very difficult to be seen by wet mount preparation, 51 stool samples showed no parasites detection by both methods .Both methods were agreed that no detection of intestinal protozoa, while by wet mount preparation 19 cases of stool samples showed the detection of intestinal parasites namely *Entamoeba coli* cyst (6 *Giardia Lamblia* cyst (6 *Hymenolepis nana* ova (3 *Entamoeba histolytica* trophozoite (3 ,*Entamoeba coli* trophozoite (1), but were showed no parasites detected by the modified ZN-stain. In this result we showed the importance of wet preparation for the detection of *Entamoeba histolytica* and *Entamoeba coli* trophozoite motility from fresh stool samples. Therefore no significant correlation was observed between wet mount preparation and modified ZN-stain.

**Table 5:** Comparison between formal ether concentration technique and trichrome stain technique

| Total | trichrome stain |      |       |              |
|-------|-----------------|------|-------|--------------|
|       | + ve            | - ve |       |              |
| 50    | 6               | 44   | - ve  | formal ether |
| 21    | 16              | 5    | + ve  |              |
| 71    | 22              | 49   | Total |              |

44 stool samples showed no parasites detection by both methods. 6 stool samples were examined microscopically by formal ether concentration technique were showed no parasites detection, while the same 6 stool samples when we examined microscopically by trichrome stain were showed the detection of *Entamoeba coli* cyst (3) ,(*Entamoeba histolytica* trophozoite (2) ,(*Entamoeba coli* trophozoite (1), This explain the importance of using trichrome stain for the detection of active infection as well as mixed infections and follow up of the parasitic infection when the number of the parasites were few and very difficult to be detected by formal ether concentration technique. Both methods were agreed in the detection of intestinal protozoa in 16 stool samples. While 5 cases of stool samples showed the detection of intestinal parasites namely *Entamoeba coli* cyst (1) *Hymenolepis nana* ova (2) ,(*Iodamoebab butcshlii* cyst

(1) ,(*Entamoeba histolytica* trophozoite (1) by formal ether concentration technique but were showed no parasites detected by trichrome stain .The sensitivity of formal ether concentration technique as compared to trichrome stain was found to be 76.1%, and the sensitivity of trichrome stain as compared to formal ether concentration technique was found to be 72.7%. In addition, there is a highly significant correlation between formal ether concentration technique and trichrome stain.

**Table 6:** Comparison between formal ether concentration technique and modified ZN-stain technique.

| Total | ZN-stain |      |       |              |
|-------|----------|------|-------|--------------|
|       | + ve     | - ve |       |              |
| 50    | 1        | 49   | - ve  | formal ether |
| 21    | 0        | 21   | + ve  |              |
| 71    | 1        | 70   | Total |              |

One stool sample were examined microscopically by formal ether concentration technique were showed no parasites detection while the same stool samples when was examined microscopically by modified ZN-stain was showed the detection of *Cryptosporidium parvum* oocyst this explains the importance of using modified ZN-stain technique for the detection of oocyst of *Cryptosporidium parvum* in diarrhoeal stool sample. Both methods were agreed that no detection of intestinal protozoa, while 21 cases of stool samples showed the detection of intestinal parasites namely *Entamoeba coli* cyst (8 ,(*Giardia Lamblia* cyst *Hymenolepis nana* ova (2 ,(*Iodamoebab butcshlii* cyst 1(*Entamoeba histolytica* trophozoite (3 ,(*Entamoeba coli* trophozoite (1) by formal ether concentration technique but were showed no parasites detected by modified ZN-stain this explain the importance of using formal ether concentration technique for the detection light infection and follow up the parasitic infection when the number of the parasites were few and very difficult to be defected by modified ZN- stain . Therefore no significant correlation was observed between formal ether concentration technique and modified ZN-stain.

**Table 7:** Comparison between trichrome stain and modified ZN-stain technique.

| Total | ZN-stain |      |       |                 |
|-------|----------|------|-------|-----------------|
|       | + ve     | - ve |       |                 |
| 49    | 1        | 48   | - ve  | Trichrome stain |
| 22    | 0        | 22   | + ve  |                 |
| 71    | 1        | 70   | Total |                 |

P = 0.5

48 stool samples showed no parasites detection by both methods. One stool sample was examined microscopically by trichrome stain was showed no parasites detection while the same one stool sample when was examined microscopically by modified ZN-stain was showed the detection of *Cryptosporidium parvum* oocyst this explain the importance of using of modified ZN-stain technique for the detection of *Cryptosporidium parvum* oocyst in diarrhoeal stool sample .Both methods were agreed that no detection of intestinal protozoa, while 22 cases of stool samples showed the detection of intestinal protozoa namely *Entamoeba coli* cyst (10 ,(*Giardia Lamblia* cyst (6) *Entamoeba histolytica* trophozoite (4 ,(*Entamoeba coli* trophozoite (2) by trichrome stain but were showed no

parasites detected by modified ZN-stain this results we showed the importance of trichrome stain for the detection of intestinal protozoa because it was able to produce an excellent contrast between nucleus, ingested RBCs and inclusions characteristics. Therefore, no significant correlation was observed between trichrome stain and modified ZN-stain. Our findings showed the staining methods Gomori's trichrome is sensitive and specific for intestinal protozoa other than coccidian and modified ZN-stain it is specific and sensitive for intestinal coccidian parasites. These results were agreed with the previous studies in Egypt (*El-Naggar et al, 2006.; Abou el Naga, 1999*)<sup>[13, 1]</sup>, Thailand (*Waywa et al, 2001*)<sup>[29]</sup>, Sudan (*Elamin, 2001*)<sup>[12]</sup>, India (*Shetty et al, 1988*)<sup>[24]</sup>, and San Francisco (*Takahashi, 1982*)<sup>[27]</sup>.

### Conclusion

The prevalence of intestinal protozoa among the food handlers was 40%, these findings draw attention to all concerned parts to plan and regulate effective control measure. Therefore, this study recommends continuous inspection by public health workers may help to reduce intestinal protozoa among unhygienic food handlers. More the less, we recommend using trichrome stain as a complementary and confirmatory method to wet mount method for detection of *E.histolytica* trophozoite in fresh or delayed bloody diarrheal sample.

### References

1. Abou el Naga IF. Studies on a newly emerging protozoal pathogen: *Cyclospora cayentanensis*. *J Egypt Soc Parasitol*,1999;29(2):575-86.
2. Albadri MA. Intestinal parasites in Waw, Sudan: A study on prevalence and comparison of diagnostic methods. University of Gezira. Sudan. August, 1998.
3. Ali El fedl SO. Evaluation of katex test for diagnosis of visceral leishmaniasis and comparative methods for detection of intestinal parasites among those patients Gadarif state-Eastern Sudan. university of Khartoum. Sudan, 2004.
4. Andargie G, Kassu A, Moges F, Tiruneh M, Huruy K. Prevalence of bacteria and intestinal parasites among food handlers in Gondar town, Northwest Ethiopia. *J Health Popul Nutr*,2008;26:451-5.
5. Babiker MA, Ali MS, Ahmed ES. Frequency of intestinal parasites among food handlers in Khartoum, Sudan. *East Mediterr Health J*,2009;15:1098-104.
6. Babiker MA. Assessment of the use of different diagnostic techniques for the detection of intestinal parasites in food handlers, Khartoum state,Sudan. University of Juba. Sudan, 2001.
7. Bigwan SZ, Galadima EIM. Comparison of Formol-Ether, Direct Smear and Nigrosine Methylene Blue for the Diagnosis of Human Intestinal Parasites. *Journal of Microbiology Research and Reviews*,2013;1(3):34-40.
8. Cheesbrough M. *District laboratory in practice in tropical countries*. Cambridge. Cambridge University Press, 1999.
9. Cheesbrough M. *Medical laboratory manual for tropical countries*. 2nd edition. Butter worth heinemann Ltd.,1987, 200-201.
10. Eisa IM. The efficiency of different techniques in the detection of intestinal parasites in school children at Kerayab village, Khartoum state; Alziem Alazhari university; Sudan, 2005.
11. Eisa HB. *Laboratory Manual for Parasitological Techniques*, First edition. Khartoum University Press, 1985.
12. Elamin AA. A comparative study of diagnostic methods of intestinal protozoan parasites with different staining methods. University of Gezira. Sudan, 2001.
13. El-Naggar SM, El-Bahy MM, Abd Elaziz J, El-Dardiry MA. Detection of protozoal parasites in the stools of diarrhoeic patients using different techniques. *J Egypt Soc Parasitol*,2006;36(1):7-22.
14. Endris M, Zinaye Tekeste, Wossenseged Lemma. Afework Kassu. Comparison of the Kato-Katz, Wet Mount, and Formol-Ether Concentration Diagnostic Techniques for Intestinal Helminth Infections in Ethiopia. *ISRN Parasitology*, 2013. Article ID 180439, 5 pages <http://dx.doi.org/10.5402/2013/180439>
15. Francis JSP, Barrett PL. Chiodini Best Practice No 174. Best practice guidelines for the examination of specimens for the diagnosis of parasitic infections in routine diagnostic laboratories *J Clin Pathol*,2003;56:888-891. doi: 10.1136/jcp.56.12.888
16. Garcai LS. *Diagnostic medical parasitology*, 4<sup>th</sup> edition. California, ASM press, 2001.
17. Ghosh A, Mishra PP, Sharma VP. Prevalence of parasitic infestations amongst the food handlers in a city of North Eastern Region of India *Natl J Integr Res Med*,2014;5(2):15-18.
18. Hailu YM, Mulu TW, Abera B. Evaluation performance of diagnostic methods of intestinal parasitosis in school age children in Ethiopia. *BMC research notes*,2015;8(1):1-5.
19. Legesse Eshetu, Regea Dabsu, Geletta Tadele. Prevalence of intestinal parasites and its risk factors among food handlers in food services in Nekemte town, west Oromia, Ethiopia. *Res Rep Trop Med*,2019;10:25-30. doi: 10.2147/RRTM.S186723
20. Markell EK, Johan DT. *Markell and vogue's. Medical parasitology*. 8th edition. USA, W. Bsaunders company press, 1999.
21. Mehrgan Hey Dari-Hengami, Yaghoob Hamedi, Majid Najafi-Asl, \*Khojasteh Sharifi-Sarasiabi. Prevalence of Intestinal Parasites in Food Handlers of Bandar Abbas, Southern Iran. *Iran J Public Health*,2018;47:111-118.
22. Moges F, Belyhun Y, Tiruneh M, Kebede Y, Mulub A, Kassu A *et al*. Comparison of formol-acetone concentration method with that of the direct iodine preparation and formol-ether concentration methods for examination of stool parasites. *Ethiop. J. Health Dev*,2010, 24(2).
23. Saab BR, Musharrafieh U, Nassar NT, Khogali M, Araj GF. Intestinal parasites among presumably healthy individuals in Lebanon. *Saudi Med J*,2004;25:34-7.
24. Shetty N, Prabhu T. Evaluation of faecal preservation and staining methods in the diagnosis of acute amoebiasis and giardiasis. *J Clin Pathol*,1988;41(6):694-9.
25. Steven LM. *pathology and understanding disease prevention*. 2nd edition. Ph D, FIBM. Stanley thornes,2003, 188-189.

26. Surtiastuti J, Manan W. Intestinal parasites from fingernails of sidewalk food vendors. *Univ Med.*,2011;30:120-125.
27. Takahashi H. The trichrome-stained smear as a screening method for intestinal parasites: evaluation in a San Francisco Bay Area population. *Am J Med Technol.*,1982;48(6):531-3.
28. Takalkar AA, Madhekar NS, Kumavat AP, Bhayya SM. Prevalence of intestinal parasitic infections amongst food handlers in hotel and restaurants in Solapur city, India. *Indian J Public Health*,2010;54:47-8.
29. Waywa D, Kongkriengdaj S, Chaidatch S, Kowadisaiburana B, Chaikachonpat S, Suwanagool S, *et al.* Protozoan enteric infection in AIDS related diarrhea in Thailand. *Southeast Asian J Trop Med Public Health*,2001;32(2):151-5.
30. World Health Organization. Food safety and food borne illness. Geneva: WHO, 2007.
31. Zagloul DA, Khodari YA, Othman RA, Faroog MU. Prevalence of intestinal parasites and bacteria among food handlers in a tertiary care hospital. *Niger Med*,2011;52:266-70.
32. Zain MM, Naing NN. Sociodemographic characteristics of food handlers and their knowledge, attitude and practice towards food sanitation: a preliminary report. *Southeast Asian J Trop Med Public Health*,2002;33:410-7.