Original Article **Sudan Medical Laboratory Journal**

A comparative study of the use of formal ether concentration, wet preparation, and zinc sulphate flotation as methods for diagnosing intestinal parasites. Omdurman, Sudan

Jamal Yousef Lowaty Lowba[1]*, Mohamed Abdurrahman[1], Ali Adam Artmby[1], Mazen Ahmed[1], Mohamed Ebrahem Suleiman[1], Ismael Abu-Bakr Ismael[1], ,Hozeefa Hasaean Alnwor[1], Mustafa Hassan Ahmed[1] and Mohanad Elhadi elfadol[1].

1. Omdurman Islamic University, Faculty of Medical Laboratory science. Omdurman -Sudan

Abstract

Background: Fecal formal ether concentration technique has become routine methods as a part of complete parasite examination. It allows the detection of small organisms that may be missed by using zinc sulphate flotation and direct microscopy techniques.

Objective: To compare the reactivity of formal ether, zinc sulphate flotation, and direct microscopy techniques in detecting parasites ova, cysts, and larvae in stool samples.

Material and Methods: This is a descriptive cross sectional study conducted during the period from September to December 2021 in Ombada hospital. A total of 50 stool samples was collected and preserved in 10% formal saline. All samples were examined using wet preparation, Zinc sulphate flotation and formal ether concentration techniques.

Results: The Positive detection rate by Zinc sulfate flotation technique (28%) and types of parasites detected *E. histolytica* (29%), *Giardia. lamblia* (25%), and the detection rate by formal ether concentration technique detection rate (40%) and The most intestinal parasites type detected *E. histolytic a* (34%), *Giardia lamblia* (43.7%) *Ascaris.lumbericoide* (0%), *H. nana* (33%) and *Taenia SPP* (50%) and wet preparation detection rate (36%) the types of parasites detected *E. histolytic* (34%), *Giardia. lamblia* (31.3%),

Key words Comparison, reactivity detection, formal ether, Zinc sulphate ,flotation Techniques.

Corresponding author: Jamal Yousef Lowaty Lowba,E-mail: Jamal yousif 120@gmail.com/

Introduction:

Stool examination using conventional techniques or commercial kits which have low sensitivity (1). Are characterized by ease of performance and low cost for direct identification of infective agents (2). Fecal formal ether concentration technique has become a routine procedure as a part of the complete parasite examination. It allows the detection of small organisms that may be missed by using only direct wet smear (3). The zinc sulphate flotation technique is commonly used in laboratories to float parasite eggs and cysts in stool specimens (4). And remain the most performed,

widespread, and appropriate methods to detect human intestinal parasites (5).However, *Microsporidium* Spp size 1-2.5 µm, *Cryptosporidium* Spp size 4-6 µm, *Cyclospora* spp size 8-10 µm, and *Cystoisospora* spp size 20-30 µm are difficult to detect by the conventional parasitological techniques. A major obstacle in the detection of these parasites is the need to perform specific permanent staining techniques (6). This technique also provides certain advantages including less alteration to organisms and increased recovery of helminthes eggs and protozoan cysts (7). That require specialized personnel or the time-consuming and labor intensive (8). In addition, errors in test interpretation can be occur because the parasitic structures (oocysts) are much smaller than larvae and helminth eggs. Therefore, clinicians may easily mistake real oocysts for yeasts or debris (9) Except for *Cysts isospora* spp. The technological development and combining alternative methods have improved the diagnostic accuracy of these tests. For instance, the parasitological examination unfortunately, extremely flammable, is highly volatile, produce anesthetic vapor, and forms explosive peroxides when exposed to light (10). Moreover, it can be a possible cause of mutagen, if inhaled or absorbed through skin with harmful long term health effect like neurotoxicity or cancer (11). The most commonly used parasitological diagnostic methods for detection of intestinal helminthic and protozoan infection in human are inexpensive and simple to perform, however they have important limitations, particularly regarding their sensitivity. Therefore, the use of more than one diagnostic methods is necessary to detect different parasitic evolving forms, such as eggs, larva, cysts, oocysts and trophozoites, due to the differences in size, morphology, density, and motility among them. More over the use of different parasitological methods is necessary to improve sensitivity for helminth and protozoa diagnosis in patients with low parasite burdens (12). This study aim to compare the reactivity of formal ether, zinc sulphate flotation, and direct microscopy techniques in detecting parasites ova, cysts, and larvae in stool samples.

Material and Methods:

This is a descriptive cross sectional study was conducted in Ombada hospitals in Omdurman-Sudan from September to December 2021. A total of 50 faecal samples was collected in containers contain 10% formal saline as preservative reagents. were examined by microscope, formal ether concentration and zinc sulphate flotation techniques as intestinal diagnostic methods, preserved faeces in formal saline were emulsified and homogenized on one drop of emulsified faecse suspensions, placed on a slide microscope, covered with cover class and examined microscopy by wet preparation technique, using the 10 x objective and 40x objective of microscope lens for detecting intestinal parasites in faeces. Using formal ether concentration method, 1 g of faeces emulsified in 4 ml of 10% forma water in test tubes, 3-4 ml of formal water added further in test tubes and mixed well, emulsified faeces sieved in beaker, 2-3 ml of diethyl ether added to

the suspension and mixed in the test tubes, homogenate suspension was centrifuged at 750-1000 g for 1 minute and the sediment was examined microscopically by using the 10x objective and 40 x to identify intestinal parasites in faeces. Zinc sulphate solution concentration was 33%w\v Reagent, specific gravity 1.180-1.200, about one quarter of zinc sulphate solution added in to test tube mixed by 1gram of faeces (or2 ml if a fluid specimen), Using a rod or stick, emulsify the specimen in the solution, Fill the tube with the zinc sulphate solution and mix well, strain the faecal suspension to remove large faecal particles, Rectum the suspension to the tube and Stand the tube in completely vertical position in a rack. Using a plastic bulb pipette or Pasteur pipette add further solution to ensure the tube is filled to the brim. Carefully place a completely clean (grease –free) cover glass on top of the tube. avoid trapping any air bubbles, lave undisturbed for 30-45 minutes to give time for the cyst and egg to float, carefully lift the cover glass from the tube by a straight, pull up wards and examine microscopically using 10x and 40 x to identify intestinal parasites.

Results: A total of 50 faecal samples collected from Ombada hospitals Omdurman examined the detection rate by the three methods for intestinal parasites shown in (Table1) and Figure (1). The positive detection rate of the formal ether Concentration technique method showed the highest rate of positive detection among this three methods the positive detection counted 40 out of 50 samples, counted (80%) On the other hand the wet preparation technique comes in second which counted 36 out of 50 represented (72%) and Zinc Sulphate solution techniques comes the third one with positive detection rate counted 28 out of 50 represent (56%). The types of parasites detected by three methods, *Endameba*. histolytic a was the most frequent detected parasite, it was more detected by wet preparation method showed detection rate 17 out of 47 represent (36.2%) and formal ether concentration technique showed detection rate 16 out of 47 represent (34%), while zinc sulphate flotation technique showed lowered positive detection rate for *E. histolytica* by rate 14 out of 47 represent (29.8 %) as shown in the (Table 2) and figure (2). *Giardia*. l*amblia* was more detected by formal ether concentration technique showed detection rate 21 out of 48 represent (43.7%), while detection rate by Wet preparation reached (31.3%) from a total 15 out of 48 and zinc sulphate flotation technique showed lowered detection rate of *Giardia. lamblia* (25%) represent 12 out of 48 in the (Table 2) and figure (3). Other parasites like *Hymenolepis nana*, *Enterovirus. vermicular*, *Taenia spp* and *Ascaris .lumbericoid* showed lowered detection rate and was more detectable by the wet preparation method as shown in the (Table 2) and figure (2).

Table 1: Positive detection rate according to type of method used.

	Detection Method	Positive		Negative		Total	
		N	%	N	%	N	%
1	Wet preparation	36	72%	14	28%	50	100%
2	Concentration Technique	40	80%	10	20%	50	100%
3	Zinc Sulphate technique	28	56%	22	44%	50	100%

Table 2: Positive detection rate according to type of parasites and methods.

	Parasites	**Wet preparation**		**Concentration technique**		**Zin sulphate technique**		**Total**	
		N	**%**	**N**	**%**	**N**	**%**	**N**	**%**
1	E. histolitica/coli	17	36.2%	16	34%	14	29.8/%	47	100%
2	Giardia. Lamb Lia	15	31.3%	21	43.7%	12	25%	48	100%
3	Ascaris .lumbricoides	1	50%	1	50%	0	0	2	100%
4	Hymenolepis .nana	1	100%	0	0	0	0	1	100%
5	Enterobius vermicalaris	1	33.3%	1	33.3%	1	33.3%	3	100%
6	Taena Species	1	50%	1	50%	0	0	2	100%

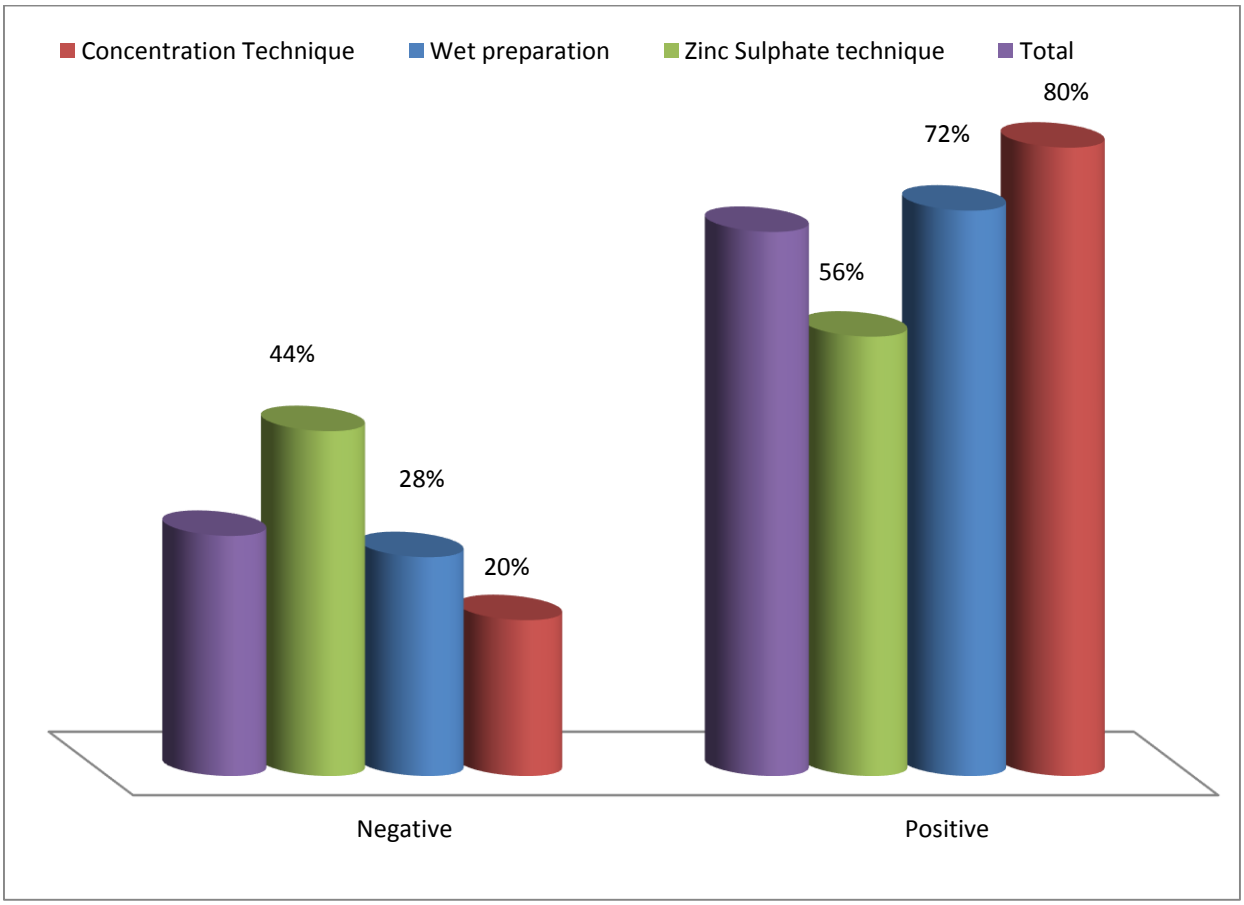

Figure 1: positive detection rate by type of methods

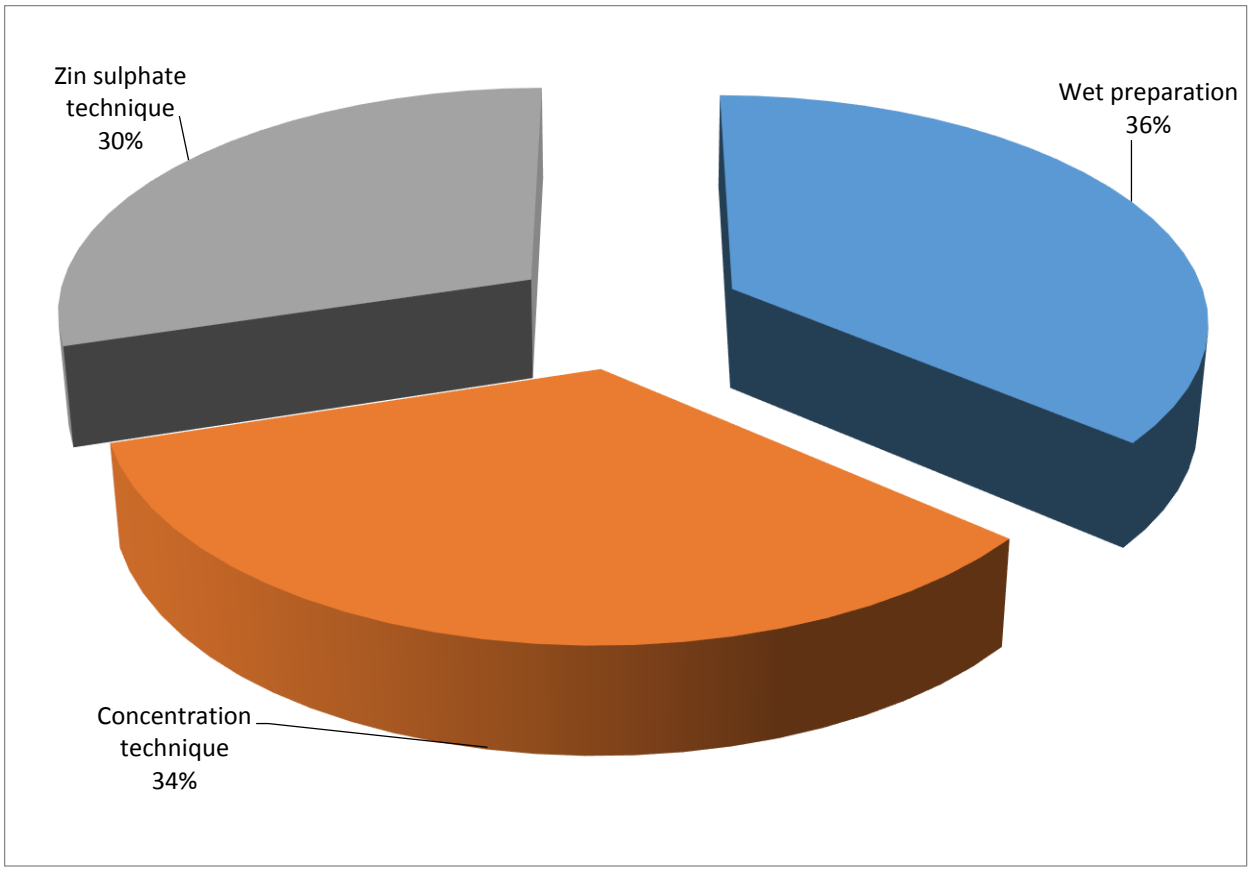

Figure 2: E. histolytic a detection rate by methods

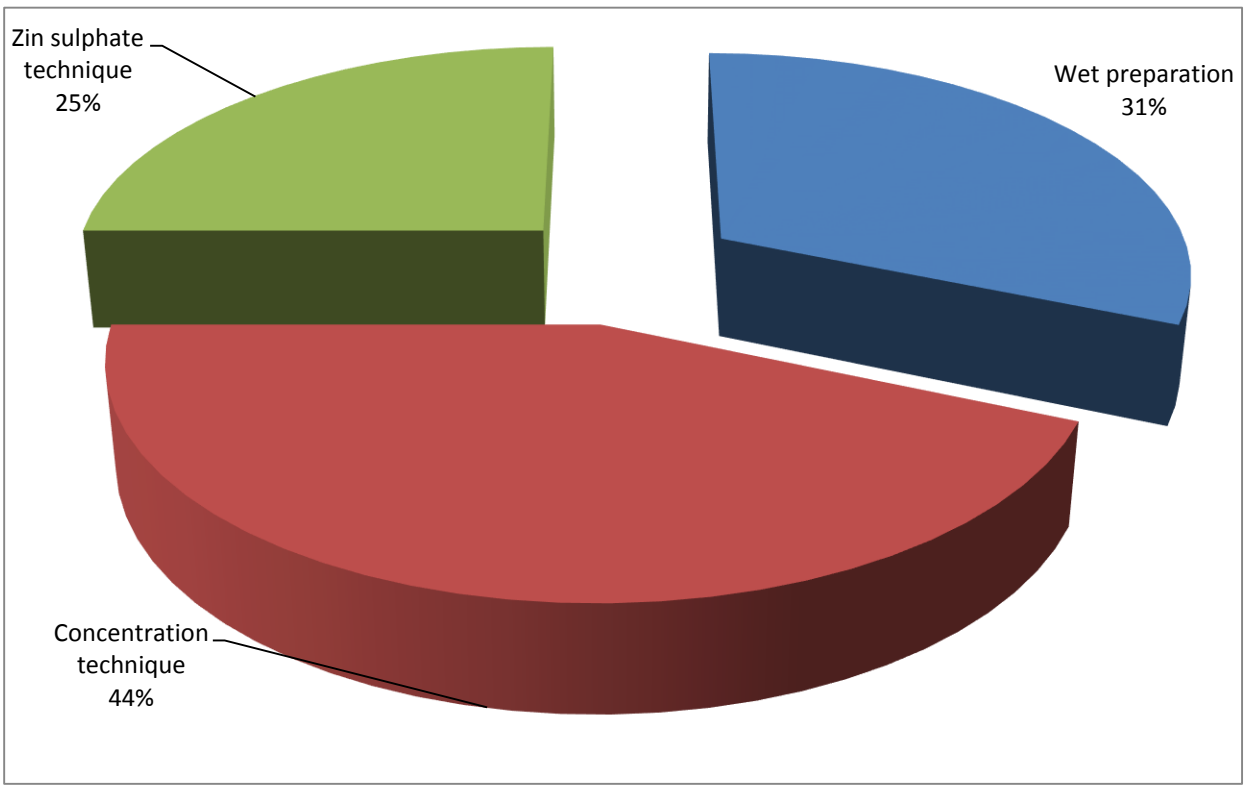

Figure 3: *Giardia*.lamblia detection rate by three methods

Discussion:

The goal in using the formal ether concentration method is to improve the detection of protozoan cysts and some helminths eggs that is missing in conventional techniques used in stools analysis by wet preparation and zinc sulphate flotation, some parasites their densities are higher than zinc sulphate and they cannot rise to the surface (13).Therefore the use of more than one parasitological methods is needed to detected different intestinal parasitic evolving forms such as eggs, larva, cyst and trophozoites due to their different in size, morphology, density and motility among them{ 14 }. In the present study the results showed that 50 stools samples examined ,six intestinal parasites detected by different methods employed the results reveal that the detection rate of protozoan parasites by formal ether concentration technique 40(80%) , wet preparation 36(72%) and zinc sulphate 28(56%) this detection rate showed significant differences in percentage between three methods used to identification of intestinal parasites when compared this results by pervious results reported by Katagiri G,etal . 2010(16) showed agreement with our results. Our results obtained in the present study showed that the frequency of protozoan parasites identified by three methods *Giardia. lamblia* counted 15/48 (31. %) by wet preparation ,21/48 (34%) formal ether concentration technique and 2/ 48 (25%) by zinc sulphate floatation technique this results showed that formal ether concentration technique and wet preparation technique were more effective techniques for identification and detection of *Giardia. lamblia* in stool samples than zinc sulphate technique which showed low accuracy in detection of *Giardia*. *lamblia* this can be explained that the hypertonic zinc solution may not be appropriate for the detection or the polymorphism of this protozoa with different micrometers when compared this results with pervious study results by Eymael D, *etal*.2010 (7) showed concordance. In The present study the frequency of *E. histolytica* detected by three methods employed showed higher frequency differences in diagnosis when detected by formal ether concentration method represent 16 out of47 (34%), wet preparation method counted 17out 47(36%) and zinc sulphate counted 14 out47 (29.8%) this results when compared by previous results reported by faust EC,*etal* .1939 (8) showed agreement. The results reveal that the heavy helminthes eggs such as those *Taenia spp* , *H.nana* and *E.vermicularis* were only detected by wet preparation method and formal ether concentration method due to their high density and subsequent tendency to settle along with various faecal debris , this results when compared with previous results reported by Scandrett WB, *etal***.**2004 (18) showed agreement. Other previous studies reported by Santos FLN, etal.2007 (17) agree with our present study results, the formal ether concentration method is a more effective technique for identification of *H.nana* and Giardia *lamblia* cyst and some eggs of helminthes in faecal samples. In our present study we observed that detection of *Taenia spp, E. vermicularis and*

H.nana diagnosed by different three methods showed significant differences detection rate in all methods because this heliminthes itis usually based on the recovery of typical eggs on perianal skin when compared this results with previous results reported by John DT, Petri WA, 2009 (20) showed agreement .

Conclusion: the prevalence of intestinal parasites diagnosed by these techniques showed that detection rate of intestinal protozoal parasites was higher significant detection rate than intestinal helminthic parasites infection, there were significant differences between these methods in the identification of intestinal parasites and formal ether concentration techniques consider to be the most accurate methods of chose in detection of intestinal parasites than wet preparation and zinc sulphate.

Conflict of interest: There are no conflict of interest.

Financial: This study has not received financial support.

Acknowledgment: We would like to appreciate laboratory staph of Ombada hospitals Omdurman.

Reference:

1. Ahmed SA, Karanis P. (2018). Comparison of current methods used to detect Cryptosporidium oocysts in stools. Int J Hyg Environ Health 221(5):743-63.

2. Amato Neto VA, Alarcón RS, Gakiya E, Bezerra RC, Ferreira CS, Broz LM. Blastocysts (2003): Controvérsias e indefinições. Rev Soc Bras Med Trop .36:515-

3. Boswell MV, Collins VJ (1996). Diethyl ether and chloroform Collins VJ, eds. Physiologic and pharmacologic bases of anesthesia. Pennsylvania, Williams & Wilkins,, 650-62.

4. Cheesbrough M (2000). District laboratory practice in tropical countries, 3rd ed. Part One, Cambridge University Press, United Kingdom, 214-215.

5. Cringoli G, Rinaldi L, Maurelli MP, UT zinger J(2010). FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat Protoc. 5(3):503-15.

6. Erdman D, Schuh GM, Tavares RG (2010). Clinical Comparison of Ethyl Acetate and Diethyl Ether. rev Soc Bras Med Trop; 43:309-12.

7. Eymael D, Schuh GM, Tavares RG (2010). Padronização do diagnóstico de *Blastocyst is* spp. por diferentes técnicas de coloração. Rev Soc Bras Med Trop 43:309-12.

8. faust EC, Sawitz W, TobieJ, Odom V, Peres C, Linicomm DR (1939). Comparative efficiency of various technique for the diagnosis of protozoa and helminth in feces. j parasitol. 25:241-62.

9. Garcia LS, Arrowood M, Kokoskin E, Paltridge GP, Pillai DR, Procop GW, (2000) . Laboratory Diagnosis of Parasites from the Gastrointestinal Tract. Clin Microbial Rev. 2018;31(1): 25-17.

10. Garcia LS ,(2010). Clinical Microbiology Procedures Handbook. American Society for Microbiology Press; p. 2265.

11. Garcia LS, Shimizu RY, Bernard CN (2000). Detection of Giardia lamblia, Ent amoeba histolytic a/Ent amoeba dispar, and

Cryptosporidium parvum antigens in human fecal specimens using the triage parasite panel enzyme immunoassay. J Clin Microbial. 38(9):3337-40.

12. Graham DF (1941). Advice for the diagnostic on the Enterovirus vermicular is infection. AmJ Trop Med HYg.21;150-1.

13. Levine JA, Estevez EG(1983). Method for concentration of parasite from small amounts of feces. *J Clin microbial*, 18, 786-788.

14. Truant AL, Elliott SH, Kelley MY, Smith JH(1981). Comparison of formalin-ethyl ether sedimentation, formalin-ethyl acetate sedimentation, and zinc sulfate flotation techniques for detection of intestinal parasites. *J Clin Microbial*, 13, 882-884.

15.RyanU, (1981). An ether sedimentation technique for routine stool examinations. *Bull US Army Med Dept.* 8, - 326

16. Katagiri G, Oliveira – Sequeira TC (2010). Comparison of three concentration method for the recovery of canine intestinal parasites from stool samples. Exp parasitol. ; 126:214-6

17. Santos FLN, Gonçalves M de S, Soares NM (2011). Validation and utilization of PCR for differential diagnosis and prevalence determination of Ent amoeba histolytic a/Ent amoeba dispar in Salvador City, Brazil. Braz J Infect Dis. 15(2):119-25

18. Scandrett WB, Gajadhar AA (2004). Recovery of putative taeniid eggs from silt in water associated with an outbreak of bovine cysicercosis. can Vet J. 45:758-60.

19. Spencer AB, Colonna GR. (2002). Fire protection guide to hazardous materials, 13th ed. National Fire Protection Association, Quinc NFPA 3y.

20. John DT, Petri WA (2009). Markell and Voges Medical parasitology. Noida India: Saunders Elsevier. p.463.

21. ZajacAM, Johnson J, King SE (2002). Evaluation of the importance of centrifugation as a component of zinc sulphate fecal flotation examinations. J Am Anim Hosp Assoc. 38:221-4.

22. Santos FLN, Peralta RHS, Macedo HW de, Barreto MGM, Peralta JM (2007). Comparison of multiplex-PCR and antigen detection for differential diagnosis of Entamoeba histolytica. Braz J Infect Dis. 11(3):365-70.