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Original Research Article

Identification of parasitic infections in stool samples by different methods: a study emphasizing the use of concentration techniques.

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Abstract: Parasitic infections remain the major public health issue in the developing countries. The prevalence of intestinal parasitic infections varies according to the geographical locales and also depends upon diagnostic methods used in different laboratories. But most of the laboratories unable to detect parasitic infections by using routine methods like wet mount due to their compromised sensitivity and specificity. Therefore demonstration of parasitic infections from the specimen's poses a huge challenge to the clinical microbiologists. So by keeping in mind the above facts, a prospective study was conducted over the period of 11 months from January 2015 to November 2015.A total of 100 fresh stool specimens were collected in sterile containers and transported to the Department of Microbiology immediately. A total of 100 stool samples were examined, out of which 45 (45%) samples were positive for ova/cyst by wet mount after concentration method. but the positivity rate by wet mount alone without any concentration method was 36%. The most common identified parasite in our study was that of *Entamoeba histolytica* (12%) followed by cyst of *Giardia* (10%), egg of *Ascaris* (5%),cyst of *Cryptosporidium*, egg of *Ancylostoma*, egg of *H.nana* (4%each), egg of *Taenia* (3%), cyst of *E.coli* (2%), egg of *Enterobius vermicula is* (1%). So there is dire need to use reliable, economical diagnostic methods which can accurately detect parasitic infections and control its spread.
Keywords: Parasitic infections, stool samples, Concentration method

INTRODUCTION:

Parasitic infections remain the major public health problem in the developing countries like India. In developing countries the high burden of parasitic infections are due to overcrowding, malnutrition, improper disposable of sewages and non-availability of potable water and unhygienic conditions [1]. The effects caused by parasitic diseases may vary from mild discomfort to severe. Some parasites may lead to anaemia and protein malnutrition which can be the cause of growth retardation in children. The prevalence of intestinal parasitic infections varies according to the geographical locales and also depends upon diagnostic methods used in different laboratories [2, 3].

The demonstration of medically important parasites poses a judicious challenge to the clinical microbiology laboratories associated with health care facilities .The routinely used methods for the detection of Ova/cysts in diagnostic laboratories are wet mounts like saline wet mount and iodine wet mount. But the sensitivity of this method is compromised. Result of this compromised sensitivity is false negative results which affects the treatment adversely and leads to worsening of symptoms and spread of infection in the community. This problem of sensitivity can be solved to better extent by using concentration techniques of the stool for demonstration of ova/cyst. Because concentration techniques can detect parasites which are present in small numbers which can be missed easily by using direct wet mounts[3]. Various concentration techniques are available and can be used for the detection of the parasitic infections depending upon availability of resources. So there is dire need of simple, economical, reliable and sensitive diagnostic methods for the detection of these parasitic infections.

By keeping in mind the above facts, we have planned a study with following objectives:

- To detect parasitic infections by using saline and iodine wet mount before and after applying concentration technique.

- To see the effect of concentration technique for the detection of ova/cyst from stool samples over wet mount alone without concentration techniques.

MATERIALS AND METHODS

A prospective study was conducted over the period of 11 months from January 2015 to November 2015.A total of 100 fresh stool specimens were collected in sterile containers and transported to the Department of Microbiology immediately. The stool samples which were contaminated with the patient's urine were rejected. Both the formed and the unformed stools were examined freshly.

Each stool specimen was examined by the following techniques.

1. Macroscopic examination: The colour, consistency, presence of blood and mucus were noted. The stool specimens were examined for the presence of worms like segments of *Taenia*, adult Hookworm, round worm either with the naked eye or with the aid of a hand lens [4].

2. Direct microscopic examination by using saline and iodine preparations: On a microscopic slide, a small amount of stool sample was emulsified in 1-2 drops of saline and iodine solution. A cover slip was placed on it by taking care that the preparation was free of air bubbles and macroscopic debris [5].

3. The microscopic examination after concentration technique: [4].

Simple salt floatation: About half tea spoon of fresh stool was placed in a flat bottomed container of less than 1.5 inches diameter and 20 ml capacity. Then, few drops of saturated salt solution is added and stirred to make a fine emulsion. More salt solution is added with stirring throughout to fill the container up to the brim, until a convex meniscus is formed. A glass slide is carefully laid on the top of the container so that the centre is in contact with the fluid. Preparation is allowed to stand for 20 minutes after which the glass slide is quickly lifted and observed for the presence of eggs/cysts

RESULTS:

A total of 100 stool samples were examined, out of which 45 (45%) samples were positive for ova/cyst by wet mount after concentration method. but the positivity rate by wet mount alone without any concentration method was 36%. So we were able to detect nine more positive samples with the mere addition of one simple concentration technique. Overall, the detection of parasitic infections was 45% in the present study.

Table-1: Rate of parasites identification before and after concentration of stool.

Name of the parasite	Number of Parasites identified	
	Without concentration	After concentration
Cyst of *Entamoeba histolytica*	09	12
Cyst of *Entamoeba coli*	2	2
Cyst of *Giardia*	08	10
Cyst of *Cryptosporidium*	4	4
Egg of *Ascaris lumbricoides*	3	5
Egg of *Ancylostoma duodenale*	3	4
Egg of *Taenia*	2	3
Egg of *H.nana*	4	4
Egg of Enterobius vermicularis	1	1
Total	36	45

DISCUSSION:

Parasitic infestations are the major causes of morbidity and mortality in developing countries like India. This is actually the area to heed but most of the time it remains neglected. Various studies have shown different prevalence rates of the parasitic infestations in different parts of India. The most common identified parasite in our study was that of *Entamoeba histolytica* (12%) followed by cyst of *Giardia* (10%), egg of *Ascaris* (5%),cyst of *Cryptosporidium*, egg of *Ancylostoma*, egg of *H.nana* (4%each), egg of *Taenia* (3%), cyst of *E.coli* (2%), egg of *Enterobius vermicularis* (1%). In studies done by Parameshwarappa KD *et al.;* Bisht D *et al.;* and Marothi Y *et al.;* also demonstrated *Entamoeba histolytica* the most common parasite [1, 6, 7]. In another study done by Srihari et al showed *E.histolytica* was the common parasite followed by *Cryptosporidium* and *Giardia* [8]. In one another study done by Kang *et al.;* commonest parasitic infection was hookworm followed by *Giardia* and *Cryptosporidium* [9]. So prevalence of different ova/cyst differs from area to area. But the main concern is about diagnosis of the parasitic infections by demonstration of parasites which is challenging for most of the laboratories. The reason behind this difference is that, for proper diagnosis, laboratories have to follow battery of tests like different wet mounts and concentration techniques. This approach requires skills, time and manpower. The routine diagnostic procedures like wet mount

examinations which are routinely followed in the laboratories lack sensitivity. So concentration methods should be done routinely for examination of stool samples for parasitic infections. The various concentration techniques like simple salt floatation, Zinc sulphate centrifugal floatation, formal-ether concentration and modified formal ether concentration techniques are available but sensitivity is different for all the techniques. In our study we have applied simple salt floatation technique; by this technique we were able to diagnose nine more positive samples as compared to wet mount examination alone. So application of concentration techniques for the detection of various ova/cyst in the stool is the need of the hour so we are strongly recommending use of concentration techniques along with wet mounts for the diagnosis of parasitic infections in stool samples in routine diagnostic laboratories.

CONCLUSION:

High prevalence of parasitic infections is the cause of concern in developing countries like India. So to fight with this public health issue, clinical microbiology laboratories should give heed to this problem of concern by using combination of methods of detection of Ova/cyst with concentration techniques of the stool that is feasible for the institute and can reliably detect parasitic infections and control its spread.

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