Gauze Filtration Technique for Quick and Inexpensive Diagnosis of Intestinal Parasites

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ABSTRACT: Intestinal parasitic infestation has been a major public health problem throughout the world, particularly in developing countries owing to unhygienic living conditions. Early and accurate diagnosis as well as improvement in detection rates of intestinal parasites is of prime importance in limiting the incidence of parasitic infestations and decreasing the disease burden. In the present study, we introduce a novel gauze filtration technique to improve the detection rates of intestinal parasites. The technique is simple, inexpensive and time-saving. Detection rate is 96.5% compared to the sedimentation technique, which was considered as the gold standard. This technique can be utilized in peripheral laboratory settings with limited resources and untrained personnel.

KEYWORDS: gauze technique, parasites, concentration methods

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Introduction

According to the World Health Organization, intestinal parasites are responsible for substantial morbidity and mortality all over the world, particularly in developing countries and especially in children. It should be emphasized that most of these infections are a consequence of the low standard of living, poverty, poor hygiene, and privation of efficient diagnostic facilities.[1,2] Infection by soil-transmitted helminths (intestinal worms) has been more frequently documented as a significant public health problem. In the 1993 World Development Report, intestinal parasitism ranked first as the chief cause of disease burden in children aged 5–14 years and also ranked highly as the disease that can be well controlled by cost-effective mediation.

Diagnosis of intestinal parasites is established by the recovery of protozoan trophozoites and cysts, helminth eggs, and larvae in the parasitology laboratory. The choice of a specific technique will be based on its affordability, simplicity to carry out, efficiency, and the level of expertise involved. Methods like DNA probes, polymerase chain reaction (PCR), and direct fluorescent antibody methods are highly sensitive but are too expensive to be used in developing countries.[3]

Routinely, microscopic examination of feces is vital in the recognition and identification of intestinal parasites. Due to the low density of parasites in feces, direct microscopy is beneficial for the observation of motile protozoan trophozoites, and the examination of cellular exudate is not suggested solely for the routine examination of suspected parasitic infections. It is crucial to improve the probability of finding the parasites in fecal samples to allow an accurate diagnosis.[4]

Concentration techniques have the aim of sorting out the parasites from fecal debris. Such techniques not only increase the number of parasites in the sediment but also unveil them, making them more noticeable by eliminating organic and inorganic debris. In most cases, diagnostic parasitology laboratories do not know the consistency of the stool, and therefore complete concentration and permanent staining are suggested.[5]

There is a critical need to improve the detection rates of intestinal parasites in populations living in extreme poverty; however, such a diagnostic technique should be economical and highly sensitive for success within the perspective of a low-income population. This study was planned to compare the sensitivity of the novel technique with the sedimentation procedure, considering latter as the gold standard, and also with the wet mount prepared directly from the fecal sample.

Materials and Methods

Fecal samples from 100 patients presenting with high eosinophil counts and/or low hemoglobin and/or retro-positivity, attending Kasturba Hospital, Manipal, and clinically being suspected of parasitic infestation were sent to the parasitology section of the Department of Microbiology, Kasturba Medical College, for investigation over a study period of 6 months. This study was exempted from seeking ethics committee approval because the patients' fecal samples were obtained and sent to the laboratory during the course of normal clinical treatment, then retrospectively utilized for research purposes.

The sample was mixed with an adequate amount of normal saline in a universal container to make a suspension. Then,

a sterile gauze piece was placed over the mouth of the container to cover it completely. The gauze piece could be folded upon itself to form two layers depending on the consistency of the fecal suspension. Another container with diameter of the mouth greater than that of the universal container was inverted and fitted over the mouth of the universal container containing the fecal suspension. Now the whole unit was inverted and the stool suspension was allowed to filter through the surgical cotton gauze piece (Fig. 1). A wet mount was prepared using the filtrate, which was then observed under the microscope under ×10 and ×40 objective lenses. If eggs or larvae were not visible, the filtrate was centrifuged at 1000 rpm for 2–3 minutes and then the supernatant was used to make a wet mount.

To compare the sensitivity of this novel method, a wet mount was also prepared by using the sediment of the fecal sample obtained by the sedimentation technique and also by preparing a wet mount directly from the stool sample. All the results were analyzed using SPSS software, ver. 16.0.

Results

A direct wet mount, wet mount using sediment of sample obtained after performing the sedimentation technique, and wet mount using filtrate of sample obtained after performing gauze filtration technique were made from each of the stool samples of 100 patients clinically suspected of parasitic infestation. It was found that parasites and/or charcot-laden crystals could be observed in 58% samples concentrated by the sedimentation technique and 56% samples concentrated by gauze filtration technique as compared to only 32% samples viewed by direct wet mount. Hookworm eggs present in 2% stool samples were missed by the gauze filtration technique, which could be observed by the sedimentation technique. All the remaining parasites that were detected by the sedimentation technique could also be detected by the gauze filtration technique.

Discussion

In 1988, a similar technique called the "spontaneous sedimentation technique in tube" (SSTT) was described by Tello[6] as a substitute to the FAUST technique (sulfate zinc flotation technique), which was then being used as the routine procedure at the Institute of Tropical Medicine Alexander von Humboldt, Universidad Peruana Cayetano Heredia (UPCH), Lima, Peru. The SSTT was shown to have greater sensitivity and required less expensive materials. A brief outline of the initial technique as described by Tello is as follows: "a 50-mL shaped plastic tube and a surgical gauze were used to filter the homogenized stools of patients infected by intestinal parasites; after filling the tube with normal saline solution and allowing it to sediment for 1–2 hours, eggs, larvae, and cysts/trophozoites of protozoa and common intestinal parasites were seen in all sediments".

In the present study, considering the sedimentation technique as the gold standard, results show that the parasite detection rate of the gauze technique was 96.5%. Compared to sedimentation technique and the gauze technique, the direct mount technique gave a parasite detection rate of 55% and 57%, respectively (Table 1). In cases where we could not find hookworm egg by the gauze technique, we performed Stoll's egg counting technique[7] and found that eggs in samples with count less than 5 could not be detected by the gauze technique. We also found that the gauze technique supplemented with vortexing the sample suspension has the same parasite detection rate as the sedimentation technique.

The novel gauze technique is based on the principle of filtration, where sterile gauze functions as a mechanical filter. It helps to remove the organic debris present in the stool sample, thereby improving the clarity of the background against which the parasite eggs/larvae can easily be appreciated. Vortexing the sample helps in concentrating the eggs, which further improves the detection rate. Filtration of organic debris and

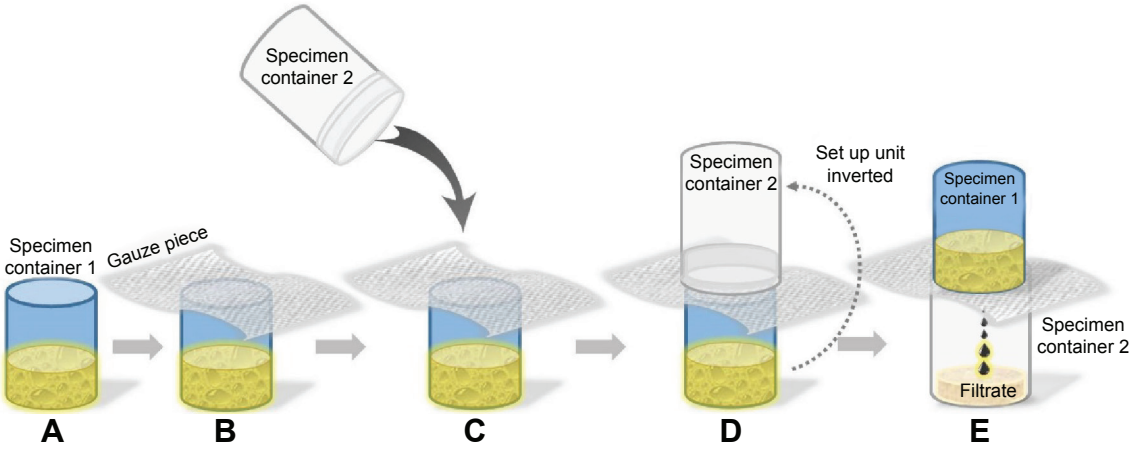

Figure 1. Schematic representations for setting up gauze technique for filtration of faecal sample (**A**). Specimen container 1 with suspension of faecal sample in normal saline (**B**). Gauze piece kept atop of container 1 (**C** & **D**). Specimen container 2 inverted over container 1 and tightly secured (**D**). The whole set up is inverted (**E**). Suspension allowed to filter through gauze and filtrate in specimen container 2 used to prepare wet mount preparation.

Table 1. Comparison of different technique for isolation of various intestinal parasites.

PARASITES ISOLATED	NO. OF SAMPLES IN WHICH PARASITES COULD BE ISOLATED BY VARIOUS TECHNIQUES ($n = 100$)		
	DIRECT WET MOUNT ($n = 100$)	GAUZE TECHNIQUE ($n = 100$)	SEDIMENTATION TECHNIQUE ($n = 100$)
E. histolytica cyst	0	5	5
Hookworm egg	12	16	18
Giardia lamblia cyst	7	12	12
Ascaris lumbricoides egg	0	2	2
Strongyloides stercoralis larvae	0	2	2
Blastocystis hominis cyst	2	5	5
H. nana egg	2	5	5
Cryptosporidium oocyst	2	2	2
Microsporidium oocyst	2	2	2
Charcot laden crystals	5	5	5
No organism	68	44	42

concentration of the eggs/larvae, together, are responsible for the sensitivity of the technique, which approaches that of the gold standard.

There are various advantages of this novel gauze filtration technique, such as reduction in the time taken for performing the technique, which is 2 minutes, as compared to the sedimentation technique, which takes 5–7 minutes. The gauze technique requires only normal saline and surgical cotton gauze, whereas the sedimentation technique requires a large number of reagents and apparatus (formalin, saline, ether, centrifuge, centrifuge tubes, brass/tea strainer), which may not be available at peripheral settings. Ether used in the sedimentation technique is inflammable and formalin is an irritant, which makes the reagents unsafe for routine use. Both the reagents require stringent methods of storage in the laboratories. The novel gauze technique does not involve the use of these reagents. It does not require formalin, and therefore the ova and oocysts can be viewed on a wet mount without any alteration of morphology, enabling more precise detection. Lower burden of parasites can be easily missed in direct mounts. Being less time consuming and economical, the gauze technique can be performed routinely in all kinds of laboratory settings. The amount of fecal debris is decreased considerably due to filtration by the gauze, which adds to the clarity and hence allows easy visualization of parasitic eggs and larvae against a clear background. Being a simple technique, it can be carried out even by untrained personnel. This technique can also be used to make wet mounts with clear background for easy visualization of eggs, cysts, and larvae by inexperienced personnel and students under training programs. Even trained professionals can employ the technique routinely for quicker and more authentic laboratory reporting of parasitic infestations. The sedimentation technique involves the use of either expensive brass sieves or tea strainers as the filter element. Since the sieves are not disposable, there is a problem with cleaning for reuse. The system is also open, so there is a biohazard and odor issue. Our technique uses disposable surgical gauze pieces. The whole unit is covered, and therefore there is no issue of odor or biohazard to the surroundings.

Conclusion

Being simple, inexpensive, and time-saving, the novel gauze filtration technique is recommended for routine application in the parasitology laboratory especially in the peripheral settings for easier detection of parasites in fecal samples.

Author Contributions

Conceived and designed the experiments: VK. Analyzed the data: KT. Wrote the first draft of the manuscript: KT. Contributed to the writing of the manuscript: PYP. Agree with manuscript results and conclusions: VK. Jointly developed the structure and arguments for the paper: KT, VK. Made critical revisions and approved final version: CM. All authors reviewed and approved of the final manuscript.

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