

Original Research Article

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A Cross Sectional Study for Prevalence of Intestinal Parasitic Infestation by Using Saline, Iodine, Glycerol-Iodine, KOH and LPCB Wet Mount Preparations of Stool Samples From Patients Attending Aims, Dewas

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ABSTRACT

The complexity of health ailments parasites inflict on human's remains a major issue worldwide. Accurate diagnosis of a parasite is the first step to fight against these parasitism and infectious diseases. To detect, identify and determine the prevalence of parasites among patients with abdominal symptoms in and around village banger, Dewas and also to compare different methods of wet mount preparations for stool examination. Stool samples from a total of 200 patients were collected and subjected for routine macroscopic as well as microscopic examination of stool by five wet mount methods namely saline, iodine, iodine-glycerol, KOH and LPCB. Concentration methods (saturated salt flotation and formal ether sedimentation) were also performed for sample negative for direct wet mount. Overall parasitic infestation was detected in 34.5% patients. This study revealed mixed parasite infestations in 7 cases. By direct wet mount, positivity was 46, which is increased to 73 after concentration of stool sample. The prevalence of protozoan infestations was much higher than the prevalence of helminth infestations and LPCB detected all parasitic forms in higher number. This study revealed a noticeable evidence of parasitisation which demands up gradation of sanitation and better living standards. Thus a approach for the control of parasitic infestation, based on streamlined health education and improved sanitation standards, is necessary measure. The use of Lacto phenol cotton blue stain along with saline wet mounts for routine microscopic examination of stools for ova& cyst can be justified by this study.

Keywords

LPCB, Iodine-Glycerol, Lugol's iodine, KOH, Stool, parasites

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Introduction

Intestinal Parasitic infestations are endemic globally and are the greatest cause of illness and disease (Sehgal *et al.*, 2010). Most of these are transmitted through soil, their route of transmission being faecally contaminated fingers, fomites, flies, food, fluid, autoinfestation, sexual route or sometimes migrate through skin to intestine (Blaser *et al.*,

2008). Poor sanitation, substandard personal hygiene practices and scarcity of potable drinking water may contribute to the quick spread of such infestations (Celiksöz *et al.*, 2005). Tourism to exotic places and ethnic food habits has led to exposure of food borne parasitic infestations (Arora and Arora, 2007). There is necessity to educate about safe drinking water, environmental sanitation and personal hygiene. Urgent remedial steps to

prevent and control intestinal parasitic infestations should be done.

Correct identification of infesting parasite is the pioneer step to combat against parasitism. Molecular biological techniques such as DNA probes, antigen detection in faeces and PCR, which cannot be afforded by resource poor countries, stool microscopy is the only test. Wet mount preparation of stool is commonly used in stool microscopy in a microbiology laboratory for the detection of intestinal parasitic infestations (Parija and Srinivasa, 1999) stool microscopy offers many advantages like accurate diagnosis, simple and extremely economical. It is gold standard test for diagnosis of parasites infestation.

Eye strain due to examination of a large number of specimen, lack of skills in technician and many more related factors leads to under-diagnosis of parasites different morphological forms by examination of stool. Therefore more sensitive methods for detecting stool parasites are need of an hour. Considering these aspects, we planned this study to know the prevalence of parasitic infestations in patients, to identify parasitic ova/egg by using various wet mount and to identify the ideal method for demonstration of the parasitic ova/cyst.

Materials and Methods

Study design

The study was carried out in Department of microbiology, Amaltas institute of medical sciences, Dewas and is a cross sectional study.

Patient inclusion criteria

The study included 200 patients with chronic abdominal symptoms (like off and on diarrhea, flatulence, mild pain in abdomen, etc) during March 2016 to September 2016.

Specimen collection

Informed consent was taken from patients. Each participant was provided with a sterile universal collection container properly labeled with details of name, age and sex. After getting sample, it was transported immediately to the central clinical microbiology laboratory and if there was any delay, it was refrigerated (Narayan Gyawali *et al.*, 2009).

Samples processing and detection of Parasites

Macroscopic examination was done to detect presence of any worms, proglottids and larva in stool followed by Microscopic examination (wet mounts).The stool samples were examined microscopically under low and high power objective by saline, iodine, glycerol iodine, KOH and LPCB wet mount preparation. Whole slide was screened by zigzag manner (Monica, 2000).

Saline wet mount were prepared directly from fecal material or from the concentrated specimens by mixing and emulsifying small volume of stool with a drop of Normal saline (0.85%) on a glass slide and placing a cover slip over the mixture.

Iodine wet mounts were prepared by adding a small amount of stool to a drop of Lugol's iodine (Hi-Media) on a glass slide, mixing and emulsifying and placing a cover slip on the mixture.

0.25 ml of pure Glycerol (Hi-Media) was added to 99.75 ml of distilled water to prepare 0.25% Glycerol. Equal quantities of 0.25% Glycerol and Lugol's Iodine were added to prepare the final stain *Glycerol-Iodine* (Vignesh *et al.*, 2008). This is used to prepare wet mount by mixing and emulsifying small volume of stool with a drop of prepared agent.

KOH wet mounts prepared by adding, mixing and emulsifying a small amount of stool to a drop of 20% KOH on a glass slide and placing a cover slip on the mixture. Wet mount is examined after 1 hour.

Lacto phenol cotton blue (LPCB) wet mounts were prepared by mixing a drop of LPCB stain (Hi-Media) with a small amount of stool on a glass microscope slide and placing a cover slip on the mixture. It was kept in wet chamber for 5 min and then examined.

Those samples which were found to be negative by direct wet mount in saline, Iodine, Iodine-glycerol, KOH and LPCB, were subjected for concentration techniques like *Saturated salt solution method* and *Formal-ether concentration*. (Monica, 2000) Samples coming negative even by concentration techniques were reported as negative.

Data was entered in excel sheet. epi info version 7.2 was used for analysis.

Results and Discussion

In the current study; out of 200 samples processed, males and females were 112(56%) and 88(44%) respectively. Among them Compared to females 22 (25%), males 47(41.96%) had higher incidence of parasitic infestation making total of 69 (34.5%). Out of total 69 cases, 7 showed mixed infestations making parasitic forms 76 in total. By direct wet mount positivity was 46, which was increased to 73 after concentration of stool sample. 3 motile trophozoites of *Giardia* were only seen in direct saline wet mount.

Out of 76 parasitic forms detected, 44(57.89%) were protozoa and 32(42.10%) were helminthes.

Table 1 shows, younger population is more affected then elderly, although all age groups

were affected in a significant number. Males (41.96%) were more affected then females (25%) (Table 1).

From the above graph, it can be seen that, LPCB mount with 46 positive parasitic forms, is the better option for screening of stool sample, while normal saline, iodine, iodine-glycerol, KOH wet mount have positivity of 22, 23, 32, 34 respectively.

Total protozoa and helminthes detected were 27 and 19 respectively. Positivity among protozoa of normal saline, iodine, iodine-glycerol, KOH and LPCB wet mount was 14, 14, 17, 21 and 27 respectively. Positivity among helminthes of normal saline, iodine, iodine-glycerol, KOH and LPCB wet mount was 08, 09, 15, 13 and 19 respectively.

From the above graph, it can be interpreted that, after concentration, LPCB mount with 73 positive parasitic forms, remains the better option for screening of stool sample, while normal saline, iodine, iodine-glycerol, KOH wet mount have positivity of 34, 41, 53, 64 respectively. Total protozoa and helminthes detected were 41 and 32 respectively. Positivity among protozoa of normal saline, iodine, iodine-glycerol, KOH and LPCB wet mount was 14, 18, 23, 36, and 41 respectively. Positivity among helminthes of normal saline, iodine, iodine-glycerol, KOH and LPCB wet mount was 20, 23, 30, 28 and 32 respectively.

From table 2, it can be inferred that, protozoa were more common than helminthes and LPCB detected all parasitic forms in higher number.

From table 3, it can be inferred that, protozoa are more common than helminthes and LPCB detected all parasitic forms in higher number. After concentration method, motile trophozoites of *Giardia lamblia* were not seen in direct normal saline wet mount.

With an ever-expanding population resulting in overcrowding and unhygienic practices, parasites pose a serious threat that is over and above added by limited resources. Like any other developing countries, India also faces intestinal parasitic infestations as a major health problem. The prevalence in this study found to be 34.5% which is in accordance with study done by ram (Ram Bilakshan Sah, *et al.*, 2013).

In studies conducted in Indore and Ujjain which are main cities near Dewas, reported the prevalence of intestinal parasitic diseases 28.9% and 21.4% (Shailendra Singh Thakur and Vipin Todase, 2018; Marothi and Singh, 2011). In one of the studies done in children of the Kashmir valley, India, has reported that 71.2 % of the sampled population showed at least one intestinal helminth. (Wani *et al.*, 2008) Rapid industrialization, change in life style and a shift of the population from rural to urban areas have caused drop in the environmental quality (Amar *et al.*, 2002).

In our study, males were more affected than female which is consistent with study done by thakur *et al.*, in Indore (Shailendra Singh Thakur and Vipin Todase, 2018). Marothi *et al.*, showed that the infestations had female preponderance (Marothi and Singh, 2011). Different studies have shown the varying sex prevalence of the parasitic infestations. However, the sex predominance for the parasite infestation has till now not been confirmed. The reason for the male dominance in our study may be due to the daily outdoor activity rather than the sex predominance.

In our study, younger and working age group had higher infestation which was similar to various studies done globally (Shammari *et al.*, 2001; Arinola and Fawole, 1995; Ramesh *et al.*, 1991; Khyati Jain *et al.*, 2018). The higher prevalence in this age group may be due to the high contamination of soil where

children play and eat food without washing their hands and working age group over here is mainly involved in agriculture work predisposing them by contaminated soil (Wani *et al.*, 2008; Awasthi and Pande, 1997).

The diagnosis of parasitic infestations in humans requires skills to identify and differentiate them from one another. Routine diagnostic procedures have low sensitivity. There is significant increase in parasitic detection after concentration in our study.

Various studies had shown significant increase in parasitic detection after concentration methods (Parija *et al.*, 2003; Parameshwarappa *et al.*, 2012). Concentration allows the detection of the parasites which are present in small numbers, non-uniformly in stool samples and may be skipped by using direct wet mounts. However to increase the yield of results both concentration techniques (Saturated salt solution method and Formal-ether concentration) should be applied on the same sample.

The high prevalence of intestinal protozoa found in this study was in accordance with previous study conducted by Marothi and Singh (2011). This is contrary to few reports documented in other parts of India where helminthic preponderance was reported (Shailendra Singh Thakur and Vipin Todase, 2018; Wani *et al.*, 2008; Awasthi and Pande, 1997).

Our study showed the presence of *Entamoeba histolytica* to be the commonest parasite which is similar to study done by Marothi and Singh (2011) and among helminthes *ankylostoma duodenale* was commonest similar to Shailendra Singh Thakur and Vipin Todase (2018), Bisht *et al.*, (2011). Thus this study highlights the occurrence of various intestinal parasites of public health importance in population residing in this area.

Table.1 Showing age and sex distribution of infested subjects

Age Range (yrs)	Subjects	Females	Infested Female	Males	Infested Males	Total infested subjects
6-15	45	21	08(38.09%)	24	14(58.33%)	22(48.88%)
16-25	46	17	06(35.29%)	29	13(44.82%)	19(41.30)
26-35	58	24	03(12.5%)	34	11(32.35%)	14(24.13%)
36-45	22	16	03(18.75%)	06	04(66.66%)	08(36.36%)
46-55	20	08	02(25%)	12	03?(25%)	04(20%)
56-65	09	02	00	07	02(28.57%)	02(22.22%)
TOTAL	200	88	22(25%)	112	47(41.96%)	69(34.5%)

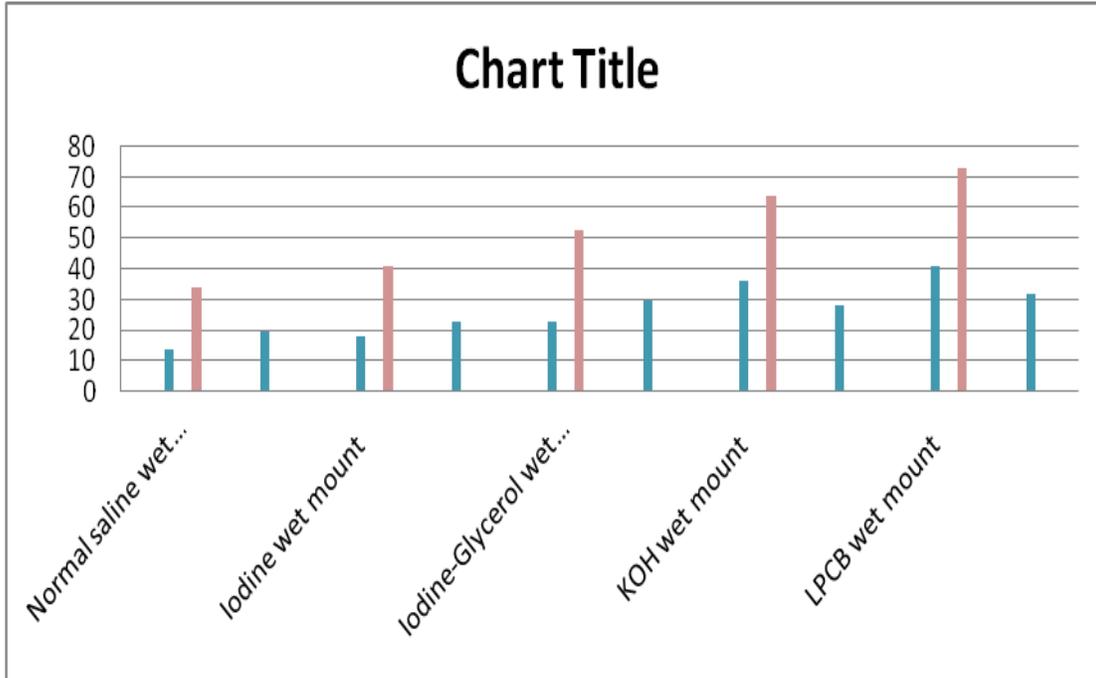
Table.2 Spectrum of parasitic forms seen in different wet mount

Parasitic form	Normal Saline Mount	Iodine Mount	Iodine-Glycerol wet mount	KOH Mount	LPCB Mount
<i>Entamoeba histolytica</i> , cyst	07	08	10	12	15
<i>Giardia lamblia</i>	04cyst + 3 trophozoite	06cyst	07cyst	09cyst	12cyst
<i>Ancylostoma Duodenale</i> , egg	04	05	07	06	06
<i>Ascaris Lumbricoides</i> , egg	02	02	04	04	07
<i>Taenia species</i> , egg	01	01	02	02	04
<i>H.nana</i> , egg	01	01	02	01	02
TOTAL	22(47.82%)	23(50%)	32(69.56%)	34(73.91%)	46(100%)

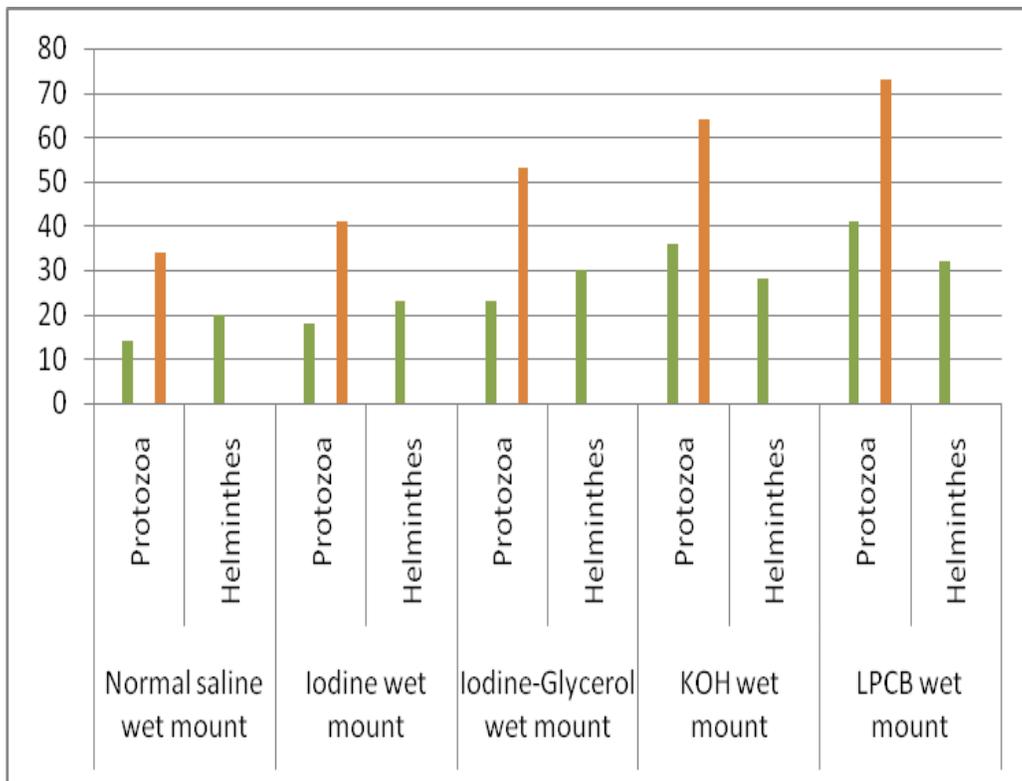
Table.3 Spectrum of parasitic forms seen in different wet mount after concentration

Parasitic form	Normal Saline Mount	Iodine Mount	Iodine-Glycerol wet mount	KOH Mount	LPCB Mount
<i>Entamoeba histolytica</i>	10	11	15	22	26
<i>Giardia lamblia</i>	04	07	08	14	15
<i>Ancylostoma duodenale</i>	10	12	15	14	16
<i>Ascaris lumbricoides</i>	05	06	08	08	08
<i>Taenia species</i>	03	03	05	04	06
<i>H.nana</i>	02	02	02	02	02
TOTAL	34(46.57%)	41(56.16%)	53(72.60%)	64(87.67%)	73(100%)

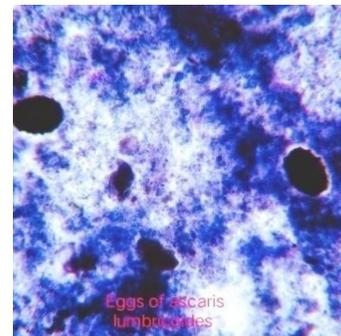
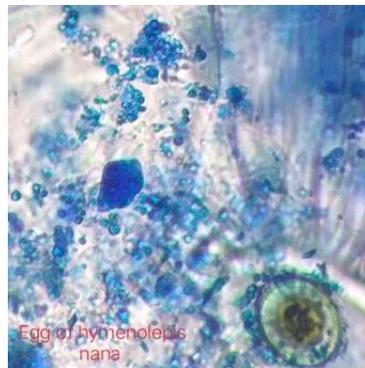
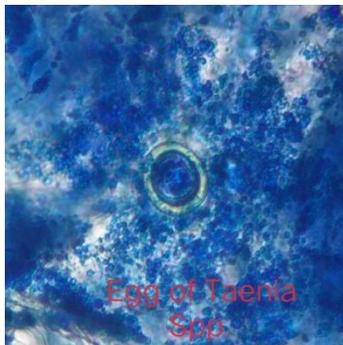
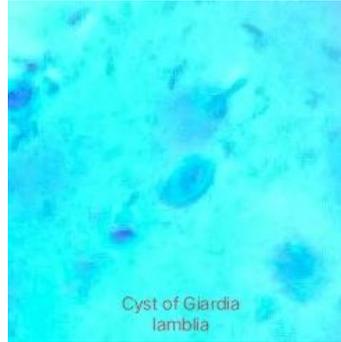
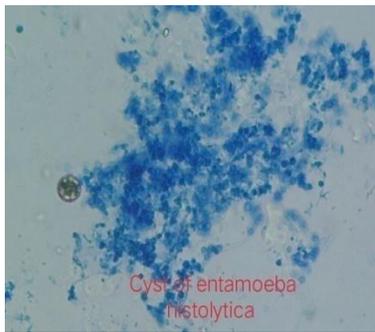
Graph.1 Spectrum of isolated parasites by different direct wet mount methods



Graph.2 Spectrum of isolated parasites by different wet mount methods after concentration of stool sample



Images showing Lactophenol Cotton Blue Wet mount Preparation in demonstrating the parasitic cyst / ova (40X)



The comparative analysis of Saline, iodine, glycerol- iodine, KOH and LPCB wet mount, LPCB wet mount showed a better positivity and better morphological characteristics.

Normal saline wet mount is the only wet mount which is able to detect motile trophozoites (Parija and Prabhakar, 1995). In our study three giardia trophozoites showing characteristic motility was found in normal saline itself.

Lugol's Iodine wet mount stains the internal structure of cyst and by increasing contrast, its detection becomes easy.

Addition of 0.25% pure Glycerol to Lugol's Iodine can potentiate the hygroscopic nature of the Glycerol-Iodine. (Vignesh *et al.*, 2008) Hence, using iodine-glycerol as an alternative to iodine wet mount can be more useful for wet mount preparations for microscopic identification of intestinal fecal parasites.

KOH wet mount in our study yield a better detection rate than Saline, iodine, glycerol-iodine wet mount. The effectiveness of KOH wet mount for intestinal parasitic detection is better because it is a clearing agent which digests proteinaceous debris and bleaches pigments without affecting the clinical materials which remain unaffected by this treatment and appear as such in the microscopic examination (Al-Doory, 1990).

Even the property of bile solubility can be visualized in KOH mount which is not possible with iodine and glycerol iodine mount. Thus KOH wet mount for examining the stool samples is simple, cost-effective and could be afforded in resource limited settings. (Rimi Farhana Zaman *et al.*, 2017) The LPCB wet mount can serve better in studying the morphology of the parasitic cyst and eggs.

LPCB lyses polymorphonuclear cells and also it stains vegetable cells, artifacts and muscle fibers (Parija and Srinivasa, 1999). LPCB wet mount stains cyst deep blue in contrast to iodine mount which stains it poorly, making the cyst difficult to overlook and the screening of stool quick (Parija and Prabhakar, 1995). Instead of iodine wet mount and KOH wet mount, LPCB wet mount can be used which has characteristic of both. Cotton blue will serve function of staining like iodine wet mount and lactic acid will clear the slide like KOH. The procedure facilitates better detection as well as identification of parasites in the laboratories where permanent stained smear of stool does not form a part of routine stool examination as LPCB wet mount can serve as semi-permanent wet mount by using DPX and can be saved for expert opinion. As LPCB stains clinically significant eggs which can be then detected quickly, decreasing time to detect them and decreasing eye strain. (Rimi Farhana Zaman *et al.*, 2017) Therefore, LPCB wet mount could serve as cost effective diagnosis of different fecal parasites.

High intestinal parasitic prevalence calls for a further evaluation of the life style, nutritional habits, and the environmental factors and making policy to educate people about hygienic practices and better living.

Concentration of stool for intestinal parasites is a useful to increase sensitivity of stool microscopy and at least one method, such as formalin-ether concentration or salt flotation method should be included as routine procedure in stool microscopy.

Our study also recommends use of normal saline along with LPCB wet mount as routine procedure for stool microscopy.

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