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Comparison of Eosin, Physiological Saline and Lugol Solutions by Using A Native Method for Detection of All Soil-Transmitted Helminths (STH)

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ABSTRACT

Stunting can be caused by worm disease because it will interfere with the absorption of nutritional intake, especially carbohydrates and protein, in children. World Health Organization (WHO) data in 2021 reported 24% of the world's population was infected by *Soil Transmitted Helminths* (STH). Worms belonging to STH are *Ascaris lumbricoides, Trichuris trichiura* and hookworms (*Ancylostoma duodenale* and *Necator americanus*). The diagnosis of intestinal worms is confirmed by microscopic examination of the feces supported by staining. This study aimed to explore and identify eosin, lugol and physiological saline and evaluate their chemical content as staining solutions to prepare stool samples. A total of 5 stool samples divided into 15 microscopic glass slide were successfully obtained from children around Politeknik Muhammadiyah Makassar. *Ascaris lumbricoides* was most prevalent helminth found in observations by all the three methods (eosin, lugol and physiological saline wet mount preparation). It was also observed that the young (1-10 years) were more infected because we took samples from childrens around the campus Politeknik Muhammadiyah Makassar. However all the three methods, eosin was found to be effective in coloring the egg stage of the STH parasites. Eosin for wet mount smear in examining the stool samples for detection of intestinal helminths may be routinely used which is simple, clarifying observations under the microscope and increasing the contrast between stained worm eggs and the background because these dye is an acidic compound that is able to fluoresce because it contains bromine and fluorescein so that it can color the cytoplasm and provide a contrast effect so that the boundaries between cells can be observed clearly.

Keywords: Eosin, Lugol, Saline preparation, STH eggs

I. INTRODUCTION

Stunting can be caused by two factors, namely internal factors and external factors. Internal factors are low intake of children, low intake of pregnant women, etc. External factor is infection in children (Benvenuto *et al.* 2022). Intestinal worm infection is one of the causes of stunting in school-age children. Soil-transmitted helminths (STH) infection is an intestinal worm infection that can significantly impact health, causing direct or indirect losses (Paun *et al.* 2021). The STHs include roundworms (*Ascaris lumbricoides*), whipworms *(Trichuris trichiura*), hookworms (*Ancylostoma duodenale* and *Necator americanus*), and threadworms

(*Strongyloides stercoralis*) (Clarke *et al.* 2019). Reports from the Ministry of Health Republic of Indonesia (2017), showed that the prevalence of the soil-transmitted helminthiasis infection was 2.5% to 62.0%. Studies have consistently reported that *Ascaris lumbricoides* is the causative agent of most STH infections, followed by *Trichuris trichiura.*

Correct identification of a parasite is the first step to ensure a combat against these parasitism and infectious diseases. The diagnosis of intestinal parasites is performed by stool examination of stools, which has been recognized as the "gold standard" for a long time (Rahmadhini and Mutiara 2015). The most commonly used methods for detecting intestinal parasites from stool examination include direct smear microscopy and concentration techniques (Moges *et al.* 2010). There are several methods for examining feces, such as direct examination (direct slide), flotation method, cover method, thick preparation technique and sedimentation method (Regina 2018).

In the examination of feces for the identification of worm eggs, it is necessary to be supported by staining. The purpose of staining worm eggs is to make it easier to study the shape of worm eggs, emphasize, and see the shape and contrast on worm egg preparations using a microscope. The dye reagent used in direct examination (direct slide) is 2% eosin which is acidic, which can provide a red background and produce a red morula color and dark red wall color in worm eggs (Oktari and Mu'tamir 2017). The physiological saline used as a staining reagent in direct smears are useful in detecting mobile trophozoite stages of Protozoa, helminth eggs and larvae. Helminth eggs, larval and protozoan cysts are also seen in the wet forms (Shyamasundari and Rao 2012). Parasitological specimen may be stained with Lugol's solution. The advantage of this method is the staining of the inner structure of worm eggs, while the disadvantage is that an excessive concentration of the Lugol's iodine solution (higher than 1% solution) causes the death of larvae of the worms, the eggs are visible, but their structure is obscured (WHO 1991). Although experienced technologists can often identify species accurately in wet mount faecal preparations, permanently stained smears are superior to saline- or iodine-stained wet mounts (Lawrence *et al.* 2004).

Research on the exploration and identification eosin, lugol and saline wet mount preparation. Additionally, the compare of staining solutions to prepare stool samples has also been widely reported. However, there have been no reports on the compare direct examination and three methods: eosin, lugol and physiological saline and their chemical content as staining solutions to prepare stool samples.

II. METHODS

Stool Specimens Collection and Processing

A total of 5 fresh stool specimens were examined miscroscopically in the Microbiology laboratory of Medical Laboratory Technology, Politeknik Muhammadiyah Makassar for the detection of intestinal parasite. Urine free stool samples were collected in a clean and leak proof, transparent container. No antiseptic or de-worming medication was give before taking these samples. Examination of specimen was performed as soon as possible, within one hour of collection. In most instances liquid stool or those specimen with mucus and or blood was examined on a priority basis (U.S. Centers For Disease (CDC) 2016).

Physiological Saline wet mount preparation

A minute portion of the faeces was diluted with physiological saline (0.9%) on a microscopic glass slide, a cover slip was then gently put over it so as to spread out the emulsion into a thin transparent layer (Zaman *et al.* 2017) and then examine the preparation under a microscope with 10x and 40x objective lenses.

Lugol wet mount preparation

Lugol wet mounts were prepared by mixing a small volume of stool to a drop of 2% Lugols iodine on the glass slide that contain saline and placed a cover slip on it and then examine the preparation under a microscope with 10x and 40x objective lenses (Sihombing and Mulyowati, 2018).

Eosin wet mount preparation

A thick stool smear was prepared by adding more volume of stool to a drop of 2% eosin on the glass object, add a small volume of stool sample and then emulsify and cover with a glass deck. Be careful not to create air bubbles. After that, it is examined under a microscope with 10x and 40x objectives (Idris and Fusvita, 2017).

Stool microscopy and Identification of worm egg/ova

The egg/ova as observed under microscope were identified by the following descriptions and pictures published by Prianto *et al.* (2008).

III. RESULTS AND DISCUSSION

Routinely submitted 5 stool samples divided into 15 glass slide were examined by the direct smear miscroscopy (in physiological saline, lugol, and eosin stains) for detection intestinal parasites. Results are presented in Figure 1 until 5.

Research on the compare of dye solutions (physiological saline, lugol, and eosin) in staining the egg stages of STH (*Soil Transmitted Helminth*) parasites obtained the results of the percentage of 2% eosin staining results obtained 100% effective results against *Ascaris lumbricoides* eggs, 0% ineffective against Hookworm eggs, and 0% ineffective against *Trichuris trichiura* eggs (Figure 1). The results of of 2% lugol staining obtained 100% effective results against *Ascaris lumbricoides* eggs, 0% ineffective against Hookworm eggs, and 0% ineffective against *Trichuris trichiura* eggs (Figure 2). The results of physiological saline obtained 100% effective results against *Ascaris lumbricoides* eggs, 0% ineffective against Hookworm eggs, and 0% ineffective against *Trichuris trichiura* eggs (Figure 3).

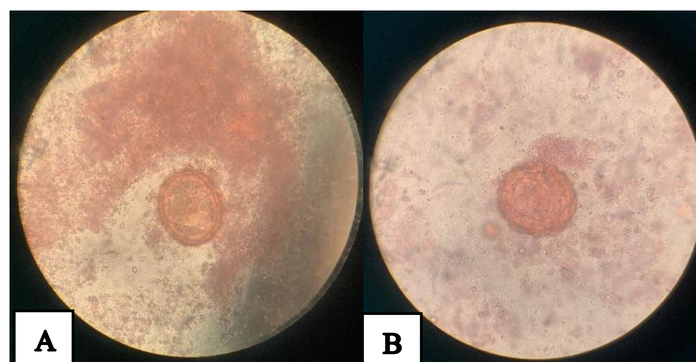

Figure 1. *Ascaris lumbricoides* decorticated egg (A) and unfertilized egg (B) with 40x objective magnification using dyes: 2% eosin

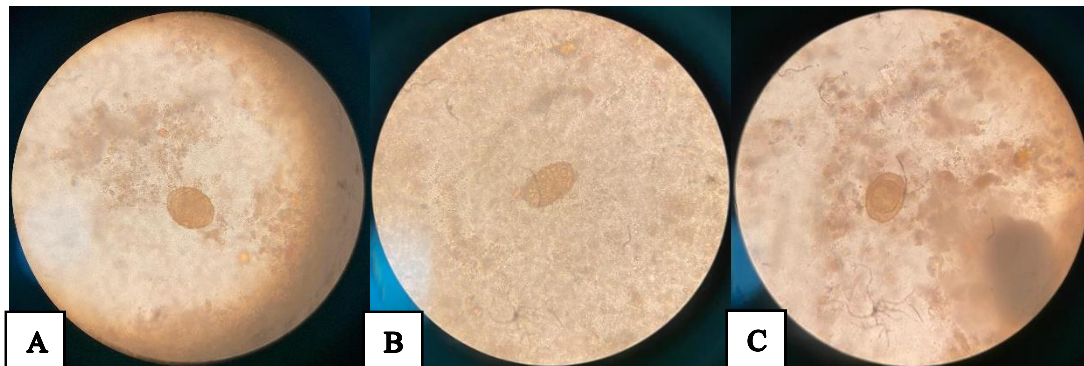

Figure 2. *Ascaris lumbricoides* fertilized egg (A), unfertilized egg (B) and decorticated egg (C) with 40x objective magnification using dyes: 2% lugol

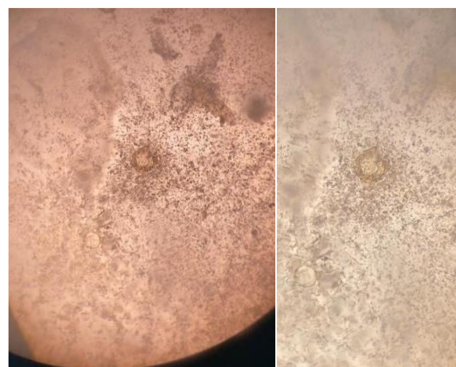

Figure 3. *Ascaris lumbricoides* fertilized eggs with 40x objective magnification using dyes: physiological saline

Physiological wet mount preparation

In the physiological saline wet preparation, the worm eggs are not stained, nonbile-stained ova of worms and so forth, are often missed due to the same backdrop in stool examinations, mostly while observing under microscope with a low-power objective (Figure 3). The chemical content of Physiological Saline Solution is natrium chloride (NaCl) around 0.9% which does not have hot properties when dissolved in water and does not have corrosive properties, this is seen in feces preparations where food residue still maintains its original form. The results of the 0.9% NaCl reagent in the preparation will look clearer and cleaner because there is no color in the reagent (Amelia *et al.* 2022).

Lugol wet mount preparation

While the Lugol's wet preparation stains the egg cells yellow-brown to brown (Figure 2). Lugol's Iodine is a rapid, non-specific contrast dye that is added to direct wet mounts of fecal material to aid in differentiating parasitic cysts from host white blood cells. Many protozoa and cysts take up the dye and appear brown while other objects in the sample remain clear. Iodine stains protozoan nuclei and intracytoplasmic organelles brown making them easier to identify. For fresh, unpreserved fecal samples, a direct wet mount should be prepared to detect the presence of motile protozoan trophozites. Make sure the wet mount preparation of the

sample has been thoroughly examined before adding Lugol’s Iodine, since iodine tends to paralyze the motility of parasitic organisms and may obscure some parasitic structures (Hastuti and Haryatmi 2021). Dilution of Lugol’s iodine is a necessity since strong iodine solutions tend to coagulate fecal particles and destroy the refractile nature of protozoan organisms (Micromaster Laboratories Private Limited 2018).

Eosin wet mount preparation

Staining using 2% eosin on *Ascaris lumbricoides* eggs gives results with a bright background so that they are easily distinguished from eggs (Figure 1). Eosin is a fluorescent, xanthene dye which binds to salts with eosinophilic compounds containing positive charges. It is the most suitable stain to combine with an alum hematoxylin to demonstrate the general histological architecture of tissues. Eosin has the ability, with correct differentiation, to distinguish between the cytoplasm of different types of cell, connective tissue fibers and matrices, by staining these differing shades of red and pink. Alternative red dyes, e.g. phloxine or Biebrich scarlet can be used as an eosin substitute but give a more intense red color to the tissues, and are rarely as amenable to subtle differentiation, making them less effective (Bancroft and Layton 2019). This indicates that Eosin 2% provides a bright colored background, clear egg shape and can be distinguished from dirt. Eosin Y (yellowish) is most widely used because it is included in acidic dyes so that it can bind to proteins that are basic and can penetrate solid structures and are metachromatic which are found in 2 forms, namely monomers (red) and dimers (orange red) (Hastuti and Haryatmi 2021). Protein is a molecule that makes up the egg layer which is basic and positively charged so that it can easily bind to eosin molecules which are acidic and negatively charged.

Stool microscopy and Identification of worm egg/ova

Morphology *Ascaris lumbricoides* fertilized eggs measure ± 60 x 45 μ, are oval, have thick walls with 3 layers and contain embryos. Unfertilized eggs measure ± 90 x 40 μ, are round or irregular in shape, have walls consisting of 2 layers and are granular inside. Decorticated eggs, eggs without an albuminoid layer that is released due to mechanical processes (Prianto *et al.* 2008). The results of the study showed that 2 samples were positive for *Ascaris lumbricoides* fertilized eggs (Figure 2 and Figure 3), 2 samples were positive for *Ascaris lumbricoides* unfertilized eggs (Figure 1 and Figure 2), and 2 samples were positive for *Ascaris lumbricoides* decorticated eggs (Figure 1 and Figure 2).

IV. CONCLUSION

Based on the results of the study, 5 stool samples were isolated from childrens around the campus Politeknik Muhammadiyah Makassar were positive for *Ascaris lumbricoides* eggs. In the present observation, it was observed that 2% eosin has a high stained rate compared to other wet mount preparations. So, the use of 2% eosin preparation along with saline wet mount preparation in a routine pathology laboratory is a good practice. Eosin wet mount is simple, clarifying observations under the microscope and increasing the contrast between stained worm eggs and the background because these dye is an acidic compound that is able to fluoresce because it contains bromine and fluorescein so that it can color the cytoplasm and provide a contrast effect so that the boundaries between cells can be observed clearly. The procedure also facilitates for better detection and identification of parasites in the laboratories.

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