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Prof. Dr Mirko Spiroski

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We have reached a decision regarding your submission to Open Access Macedonian Journal of Medical Sciences, "Synthetic Food Coloring as an Alternative to Worm Egg Staining Ascaris lumbricoides", Manuscript ID = OJS7495, submitted {submission} accepted

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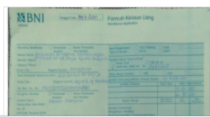
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Dear Prof. Dr Mirko Spiroski

I have transferred the regular publication fee for the manuscript " Synthetic Food Coloring As An Alternative To Coloring Worm Eggs Ascaris Lumbricoides. I also attach a publication fee transfer document. Thank you.

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Thank you for your fine contribution. On behalf of the Editors of the Open Access Macedonian Journal of Medical Sciences, we look forward to your continued contributions to the Journal.

Cordially,
Prof. Dr Mirko Spiroski,
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Synthetic Food Coloring as an Alternative to Worm Egg Staining *Ascaris lumbricoides*

AQ2,7 Steven Jacob Soenjono¹, Budi Setiawan^{2,3}, Rahmah Siti Wulandari², Suyana², Marta Atik Martiningsih²

AQ3 ¹Department of Environmental Health, Politeknik Kesehatan Kementerian Kesehatan Manado, Indonesia; ²Department of Medical Laboratory Technology, Politeknik Kesehatan Kementerian Kesehatan Yogyakarta, Indonesia; ³Department of ???, Pusat Unggulan IPTEKS Inovasi Teknologi Kesehatan Masyarakat (PUINOVAKESMAS), Poltekkes Kemenkes Yogyakarta, Indonesia

Abstract

Examination of worm eggs is a way of diagnosing helminthiasis by giving 2% eosin as a dye, but this reagent is carcinogenic so an alternative is needed to replace it. Synthetic food coloring (carmoisin CI 14720) can staining worm eggs against a more contrasting background than eosin. The purpose of this study was to determine the synthetic food coloring with a concentration of 1%, 2%, 3%, 4%, and 5% which can be used as an alternative to 2% eosin in the examination of *Ascaris lumbricoides* eggs. This type of research is observational with a cross-sectional research design. Positive stool samples for *A. lumbricoides* eggs were obtained from the Parasitology Laboratory of the University of Indonesia. Data were analyzed by ANOVA and interclass correlation coefficient (ICC) tests. The results of the ANOVA test showed that there was no difference between 2% eosin and food coloring. The results of the ICC test obtained a value of 0.845, so the dye can be used as an alternative to 2% eosin in the examination of *A. lumbricoides* eggs.

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AQ2 Citation: Soenjono SJ, Setiawan B, Wulandari RS, Suyana, Martiningsih MA. Synthetic Food Coloring as an Alternative to Worm Egg Staining *Ascaris lumbricoides*. Open Access Maced J Med Sci. 2021 Feb 05; 9(B):1-3. <https://doi.org/10.3889/oamjms.2021.7495>

Keywords: *Ascaris lumbricoides*; Carmoisin CI 14720; Eosin 2%

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AQ4 Copyright: © 2021 Steven Jacob Soenjono, Budi Setiawan, Rahmah Siti Wulandari, Suyana, Marta Atik Martiningsih

AQ5 Funding: This research did not receive any financial support

AQ2 Competing Interest: The authors have declared that no competing interest exists

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Introduction

One-third of the world's population is infected with at least one species of soil-transmitted helminths (STHs) with *Ascaris lumbricoides* infecting 800 million people, *Trichuris trichiura* 600 million, and hookworm 600 million and causing up to 135,000 deaths annually [1]. STHs have also been classified among the most common neglected tropical diseases because they persist exclusively in the poorest populations (i.e., US\$1.25/day) and are thus often overlooked [2]. Individuals infected with STH may suffer from anemia, stunted growth, reduced physical fitness, and impaired cognitive development [3] which show a continued decline in socioeconomic development in low-income countries [4], [5].

At present, the identification of parasitic worms is more focused on examining eggs as the basis for determining the genus of worms, although it is generally known that many parasitic eggs are relatively the same [6]. Identification can be done in several ways, one of which is by direct (native) examination of the preparation. The dye reagent used in this examination 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 [7]. However, it is suspected that eosin can be a carcinogen in living things [8], therefore, alternative materials are needed to replace them.

One of the dyes that are easily available is synthetic dyes for food. The dye contains carmoisin dye with CI code 144,720 which produces a dark red color. Carmoisin is a synthetic dye (water soluble) containing an azo structure and an aromatic ring [9] and not carcinogenic [10]. Many researches related to alternative materials to replace eosin have been carried out, but there has been no research using carmoisin to color *A. lumbricoides* eggs. In this study, we evaluated the quality of carmoisin as a potential alternative for staining *A. lumbricoides* compared to 2% eosin.

Methods

This research is observational analytic with cross-sectional study, feces examined with direct method. Samples of *A. lumbricoides* eggs were obtained from the Parasitology Laboratory of

the University of Indonesia. The fecal sample was examined by 10 examiner to give a score with the criteria of a score of 1, the preparation had a non-contrast field of view, the worm eggs did not absorb color and the egg part was not clear, the score was 2, if the preparation had a less contrasting field of view, the worm eggs did not absorb color, and the eggs are not clearly visible, the score is 3 when the visual field is contrasting, the worm eggs absorb the color and the egg parts are clearly visible.

The data are presented in a table of each concentration then ANOVA analysis is carried out then followed by interclass correlation coefficient (ICC) analysis to determine the ICC value at each concentration to determine the reliability of the examination results.

Results and Discussion

Based on the research results shown in Figure 1, the food coloring used can color all parts of the egg, but the best concentration is at 2%. Research conducted by Nurmufidah (2020), in the description of the variation in the concentration of Giemsa solution on the quality of STH worm egg preparations, it was found that the quality of preparations with the best quality at a concentration of 3% with a constant white background, easier, and more comfortable to distinguish with worm eggs, the embryo is purplish with a purplish-blue color on the egg wall of the *Necator americanus/Ancylostoma duodenale* with 150% effectiveness so that it can be used as a substitute for 2% eosin in the manufacture of worm egg preparations by the direct method. While this study used a synthetic food coloring carmoisin CI 14720 with the results of the preparation that is a white background with pink eggs, morula can be seen clearly.

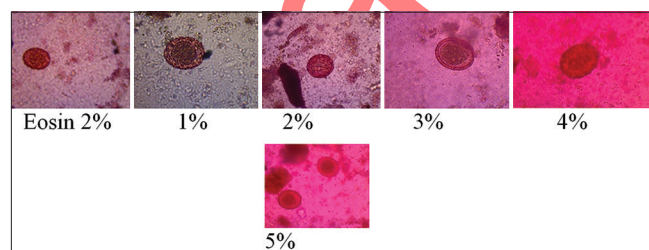


Figure 1: Microscopic view of staining worm eggs

The principle of staining on worm eggs is the affinity (formation of chemical bonds) between the tissue and the dye, either directly or indirectly [11]. Carmoisin CI 14720 dye tends to be slightly alkaline [12]. This is evidenced by the examination of the pH of carmoisin CI 44720 at each concentration having the same pH of 7.5. Carmoisin CI 14720 contains an azo structure and an aromatic ring [13] Carmoisin uses propylene glycol as a solvent. Propylene glycol functions as a humectant that will maintain the stability of the preparation by

Table 1: Carmoisin staining analysis

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Examiner	Carmoisin dye concentration					Eosin 2%	ICC
	1%	2%	3%	4%	5%		
1	1	3	3	2	3	2	0.845
2	2	3	3	1	2	3	
3	3	3	3	2	2	3	
4	3	2	3	2	2	3	
5	3	3	3	2	2	3	
6	3	3	2	2	2	2	
7	3	3	3	3	3	3	
8	3	3	3	3	3	3	
9	3	3	2	1	2	3	
10	3	3	3	2	2	3	
ANOVA test	0.102	0.645	0.286	0.242	0.486		

reducing evaporation of water from the preparation and absorbing moisture from the environment [14].

Worm eggs contain albuminoid which is a type of protein [15]. Protein molecules have an amino group (-NH₂) and a carboxylic group (-COOH) at the ends of the chain [16]. This causes the protein to have a lot of charge (polyelectrolyte) and is amphoteric, that is, it can react with acids and bases [17] then the chemical bond formed between carmoisin and protein is a carboxylic group reacting with OH⁻ ions, the presence of a charge on the protein molecule causes the protein to move under the influence of an electric field [18] so that the negatively charged protein that causes *A. lumbricoides* worm eggs can be stained by positively charged carmoisin CI 14720.

Conclusion

Carmoisin CI 14720 synthetic food coloring can be used as an alternative dye for *A. lumbricoides* worm eggs with an ICC value of 0.845. Further research can examine the effect of the synthetic food coloring carmoisin CI 14720 on the examination of worm eggs with different worm eggs.

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Synthetic Food Coloring as an Alternative to Worm Egg Staining *Ascaris lumbricoides*

AQ2,7 Steven J. Soenjono¹, Budi Setiawan^{2,3}, Rahmah Siti Wulandari², Satrio A², Marta Atik Martiningsih²

AQ3 ¹Department of Environmental Health, Politeknik Kesehatan Kementerian Kesehatan Manado, Indonesia; ²Department of Medical Laboratory Technology, Politeknik Kesehatan Kementerian Kesehatan Yogyakarta, Indonesia; ³Department of Pusat Unggulan IPTEKS Inovasi Teknologi Kesehatan Masyarakat (PUINOVAKESMAS), Poltekkes Kemenkes Yogyakarta, Indonesia

Abstract

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Correspondence: Soenjono SJ, Setiawan B, Wulandari RS, Martiningsih MA. Synthetic Food Coloring as an Alternative to Worm Egg Staining *Ascaris lumbricoides*. Open Access Maced J Med Sci. 2021 Feb 05; 9(B):1-3. <https://doi.org/10.3889/oamjms.2021.7495>

Keywords: *Ascaris lumbricoides*; Carmoisin CI 14720

*Correspondence:

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Funding: This research did not receive any financial support

Competing Interest: The authors have declared that no competing interest exists

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Introduction

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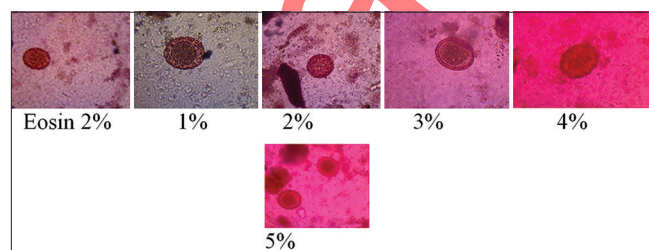


Figure 1: Microscopic view of staining worm eggs

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3	3	3	3	2	2	3	
4	3	2	3	2	2	3	
5	3	3	3	2	2	3	
6	3	3	2	2	2	2	
7	3	3	3	3	3	3	
8	3	3	3	3	3	3	
9	3	3	2	1	2	3	
10	3	3	3	2	2	3	
ANOVA test	0.102	0.645	0.286	0.242	0.486		

reducing evaporation of water from the preparation and absorbing moisture from the environment [14].

Worm eggs contain albuminoid which is a type of protein [15]. Protein molecules have an amino group (-NH₂) and a carboxylic group (-COOH) at the ends of the chain [16]. This causes the protein to have a lot of charge (polyelectrolyte) and is amphoteric, that is, it can react with acids and bases [17] then the chemical bond formed between carmoisin and protein is a carboxylic group reacting with OH⁻ ions, the presence of a charge on the protein molecule causes the protein to move under the influence of an electric field [18] so that the negatively charged protein that causes *A. lumbricoides* worm eggs can be stained by positively charged carmoisin CI 14720.

Conclusion

Carmoisin CI 14720 synthetic food coloring can be used as an alternative dye for *A. lumbricoides* worm eggs with an ICC value of 0.845. Further research can examine the effect of the synthetic food coloring carmoisin CI 14720 on the examination of worm eggs with different worm eggs.

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