

Effect of formalin concentration on the development of *Ascaris lumbricoides* eggs

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Abstract

Microscopic detection of worm eggs in fecal specimens requires preservation if the examination cannot be performed immediately. Formalin is widely used as a preservative, but its effectiveness at different concentrations in maintaining the integrity of worm eggs remains unclear. This study aims to evaluate the effect of varying concentrations of formalin on the development of *Ascaris lumbricoides* eggs in preserved stool specimens. This study used a pure experimental design with a randomized posttest-only control group. Twenty-four faecal specimens containing *Ascaris lumbricoides* eggs were treated with formalin at concentrations of 10%, 15%, 20%, 25%, and 30%, and one control group without formalin. Samples were determined using purposive sampling techniques. Egg development was observed microscopically after preservation, and the data were analyzed using a one-way analysis of variance. The results showed no statistically significant differences in egg development between all formalin treatment groups and the control group. These findings indicate that increasing formalin concentration does not enhance its effectiveness in inhibiting the development of *Ascaris lumbricoides* eggs. The commonly used formalin concentration may not be optimal for preserving fecal specimens intended for detailed morphological assessment of eggs, highlighting the need for alternative preservation strategies in routine diagnostic practice.

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1. Introduction

Intestinal nematodes include several species that require soil to complete their life cycles. One of the important species is *Ascaris lumbricoides*. This species generally infects people with poor hygiene and sanitation. This species infects many tropical and subtropical regions, including the Americas and Asia. The primary mode of transmission is faecal-oral, namely via soil, water, or food containing eggs (Chammartin et al., 2013; Dold, 2014; Mascarini-Serra, 2011; Melissa et al., n.d.; Scholte et al., 2013). Chronic *Ascaris lumbricoides* infection can lead to malnutrition, resulting in stunted growth and physical development in children. The parasite can migrate to the bile ducts, leading to colic, cholecystitis, and pancreatic inflammation. These parasites can also cause complications such as volvulus, perforation, and death (Andrade et al., 2015; Das et al., 2007; Enrique et al., 2024; Kasiita et al., 2025).

The disease caused by this species is called Ascariasis. Ascariasis can be diagnosed by laboratory examination, specifically microscopic examination of faeces (Lamberton et al., 2015; Rahmat et al., 2023). Faeces used for microscopic examination are generally preserved in 10% formalin. Formalin helps preserve

the integrity of stool samples over time, so its administration is essential to ensure the accuracy of microscopic examinations. It is essential when sample processing needs to be delayed or when the sample must be sent to another laboratory (Linscott & Sharp, 2022; Sarirah et al., 2019). Formalin acts as a fixative solution that inactivates many microorganisms. It can reduce the risk of infection and contamination during handling and examination (Linscott & Sharp, 2022; Ramesh et al., 2017). However, it should also be noted that formalin may not completely inactivate all types of microbes (Ramesh et al., 2017).

Formalin preservatives help maintain the intact morphology of worm eggs and protozoan cysts, thereby ensuring the accuracy of identification and quantification results in microscopic examinations, especially in examinations to detect Soil-Transmitted Helminths (STH) and other parasites. The addition of 10% formalin to faecal samples is handy for long-term storage without significant loss of sample quality. Studies show that the morphology of STH eggs can be well preserved for more than 12 months in formalin (Linscott & Sharp, 2022; Sarirah et al., 2019).

Ascaris lumbricoides eggs that contain a morula will develop into larvae, so that the eggs become infectious. However, it is not yet known whether formalin preservatives can prevent the development of eggs from the morula stage to the infectious stage. Therefore, this study examines whether formalin preservatives can prevent larvae from developing into infective larvae by varying formalin concentration, namely by providing formalin preservatives in the feces of Ascariasis patients at 10%, 15%, 20%, 25%, and 30%.

2. Method

2.1 Research Design and Participants

This study is a pure experimental study using a posttest-only randomized control group design. This design was used to assess the effect of varying concentrations of formalin on the development of *Ascaris lumbricoides* eggs after treatment. The study population consisted of fecal specimens from residents of Kilasah Village, Serang District, Banten Province, which were identified as containing *Ascaris lumbricoides* eggs. Sampling was conducted purposively with inclusion criteria of fresh feces containing *Ascaris lumbricoides* eggs at the morula stage. A total of 24 fecal samples met the criteria and were used in this study. The samples were then divided into six groups: five treatment groups with different formalin concentrations and one control group without formalin.

2.2 Instruments and Data Collection

Sampling was conducted in Kilasah Village, Serang District, Banten Province, while all laboratory testing was carried out at the Parasitology Laboratory of the Health Analyst Study Program, Polytechnic of the Health Ministry of Health Jakarta III. The specimens used were fresh feces excreted within the past 24 hours and were preliminarily examined and found to contain *Ascaris lumbricoides* morula-stage eggs. Each stool sample was divided into sterile sample bottles, with 4 bottles per group. The treatment groups received formalin at concentrations of 10%, 15%, 20%, 25%, and 30%, while the control group received only stool without preservatives. After formalin addition, each sample was examined microscopically daily to monitor egg development until infectious larvae were detected. The number of days required for larval formation was recorded as part of the examination data.

2.3 Data Analysis

The data obtained were the times to the infectious larval stage for *Ascaris lumbricoides* eggs in each treatment and control group. All data were first checked for completeness, then statistically analyzed using a one-way analysis of variance (ANOVA) in SPSS version 26. The test was conducted to compare the means of more than two treatment groups with numerical dependent variables. The statistical significance level was set at 0.05.

2.4 Ethical Considerations

The study did not involve human subjects directly but used biological specimens, such as feces, obtained without personal identification. The risk to individuals was negligible. All research procedures were carried out in accordance with laboratory safety principles and standard operating procedures for parasitological examination. Biological waste was handled and disposed of in accordance with biosafety regulations to prevent environmental contamination and researcher exposure.

3. Results and Discussion

3.1 Results

Sampling was conducted in the village of Kilasah, Serang, East Banten. Samples were obtained from 2 villagers with severe Ascariasis. All villagers live in poor, unsanitary conditions. Villagers defecate on the edges of rice fields or in sewers. Most villagers do not have toilet facilities at home. In the village of Kilasah, there is a pool we share. The pond is used for bathing, washing dishes and clothes, and washing foodstuffs such as rice, vegetables, and meat. The pond water is filthy, and the water comes from rivers and rice fields. For cooking, residents usually take water from the river because the village is close to the beach, so the water is healthy but salty. In the village, there are public toilet facilities, but they do not function optimally because residents prefer to defecate at the edges of rice fields or along sewer lines. Based on the survey results, it appears that villagers often sit and relax outside their homes with uncovered snacks, which are often infested with flies. Children often play on the ground without footwear. The rice fields are very close to residential houses, and if there are livestock, the house with the cattle pen is very close.

Overall, *Ascaris lumbricoides* worm eggs developed in the control and at formalin concentrations of 10%, 15%, 20%, 25%, and 30%. Development in the form of a 1-cell morula divides into a 2-cell morula, then divides into a 4-cell, 8-cell, 16-cell morula, etc., until larvae are formed. The number of days required for worm eggs to develop into active larvae in each treatment is shown in Table 1.

Table 1: Time of Egg Development of *Ascaris lumbricoides* to Infective

Replication (r)	Control (days)	Treatment (days)				
		10%	15%	20%	25%	30%
1	10	11	12	12	11	12
2	9	10	11	10	10	11
3	10	12	12	12	12	10
4	11	12	10	11	12	12

In control, the time to become infective is 9-10 days, whereas under 10%, 15%, 20%, 25%, and 30% treatment, it is 10-12 days. Based on the analysis of the substance, it can be concluded that in both the control and the treatment with formalin at all concentrations, the development of *Ascaris lumbricoides* worm eggs still occurs. To determine whether there is a significant difference between the control and the formalin-treated samples at concentrations of 10%, 15%, 20%, 25%, and 30%, statistical tests can be used.

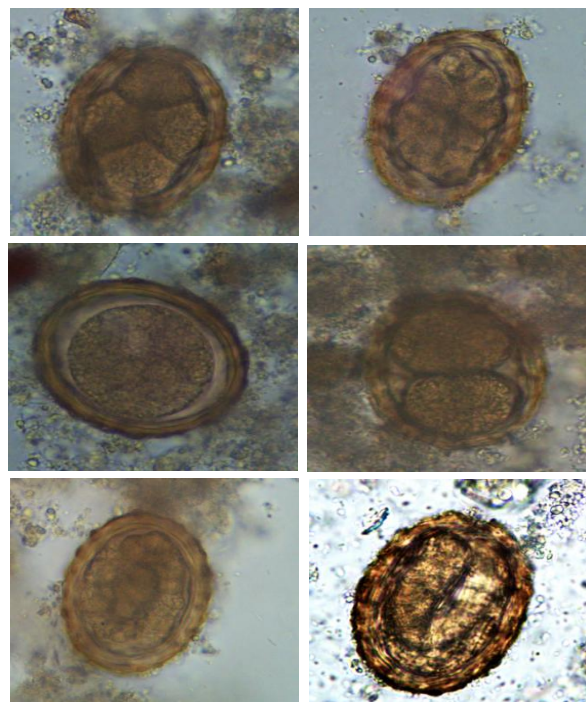


Image 1. Egg development of *Ascaris lumbricoides*

All data were analyzed descriptively. Descriptive analysis is carried out as a basis for analytical statistics (hypothesis testing) to characterize the data. Descriptive analysis was conducted using SPSS. The selection of data presentation and hypothesis testing depends on whether the data distribution is normal. The data distribution was tested for normality using the Shapiro-Wilk test at the 5% significance level. The data are normally distributed if the p-value of the normality test is greater than α . Based on the value in Table 2, the p-value is >0.05 , indicating that the data are normally distributed.

Table 2 Data Normality Test Results

Formalin concentration and control		Shapiro-Wilk		
		statistic	F	sig.
Time becomes infectious	Control	0.945	4	0.683
	formalin 10%	0.863	4	0.272
	formalin 15%	0.863	4	0.272
	formalin 20%	0.863	4	0.272
	formalin 25%	0.863	4	0.272
	formalin 30%	0.863	4	0.272

The data were tested for homogeneity of variance using *Levene's Test* (F Test) at the 5% significance level. The data is homogeneous when the P value is $>\alpha$, and the data is heterogeneous when the P value is $\leq \alpha$ —based on Table 3, $p > 0.05$, indicating that the data are homogeneous.

Table 3 Data Homogeneity Test Results

Time becomes infectious			
Levene Statistic	f1	f2	Sig.
0.214	5	18	0.952

Based on the normality and homogeneity tests, the data are normally distributed and homogeneous, so the One-Way ANOVA test is used to test the hypothesis, followed by the *Least Significant Difference* (LSD) test (treatment effect test). If the P value < 0.05 indicates a significant influence of the addition of formalin on the development of *Ascaris lumbricoides* worm eggs.

Table 4: One-Way Anova Test Results

Time becomes infectious					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.208	5	1.042	1.190	0.353
Within Groups	15.750	18	0.875		
Total	20.958	23			

Based on the results of the *One-Way ANOVA test* in Table 4, the P-value was > 0.05 , indicating that the alternative hypothesis was rejected and the null hypothesis was accepted. It shows that there is no meaningful effect of adding 10%, 15%, 20%, 25%, or 30% formalin on the development of *Ascaris lumbricoides* worm eggs. In the *Least Significant Difference* (LSD) test in Table 5, the P value was > 0.05 . The results show that there was no significant difference

Table 5. Test Results of Least Significant Difference (LSD)

Time becomes infectious LSD				
(I) Formalin and control	(J) Formalin and control	Mean Difference (I-J)	Std. Error	Sig.
Control	formalin 10%	-1.250	0.661	0.075
	formalin 15%	-1.250	0.661	0.075
	formalin 20%	1.250	0.661	0.075
	formalin 25%	-1.250	0.661	0.075
	formalin 30%	-1.250	0.661	0.075
formalin 10%	control	1.250	0.661	0.075
	formalin 15%	0.000	0.661	1.000
	formalin 20%	0.000	0.661	1.000
	formalin 25%	0.000	0.661	1.000
	formalin 30%	0.000	0.661	1.000
formalin 15%	control	1.250	0.661	0.075
	formalin 10%	0.000	0.661	1.000
	formalin 20%	0.000	0.661	1.000
	formalin 25%	0.000	0.661	1.000
	formalin 30%	0.000	0.661	1.000
formalin 20%	control	1.250	0.661	0.075
	formalin 10%	0.000	0.661	1.000
	formalin 15%	0.000	0.661	1.000
	formalin 25%	0.000	0.661	1.000
	formalin 30%	0.000	0.661	1.000
formalin 25%	control	1.250	0.661	0.075
	formalin 10%	0.000	0.661	1.000
	formalin 15%	0.000	0.661	1.000
	formalin 20%	0.000	0.661	1.000
	formalin 30%	0.000	0.661	1.000
formalin 30%	control	1.250	0.661	0.075
	formalin 10%	0.000	0.661	1.000
	formalin 15%	0.000	0.661	1.000
	formalin 20%	0.000	0.661	1.000
	formalin 25%	0.000	0.661	1.000

3.2 Discussion

The development of fertilized *Ascaris lumbricoides* eggs from the morula stage to the larval stage involves several different stages and environmental conditions. The Morula stage is the initial stage of a fertilized egg, characterized by a collection of cells. By the end of the first week of incubation, most eggs are in the final morula stage. By the 14th day of incubation, about 90% of the eggs have progressed to the larval stage (Cruz et al., 2012). Formalin is commonly used in various concentrations to preserve fecal samples for diagnostic purposes. For example, 10% formalin is used in combination with other reagents for the extraction and preservation of parasite worm eggs (Naidoo & Archer, 2024).

Formalin, particularly in higher concentrations, has been shown to inhibit the normal development of *Ascaris* eggs. For example, 5% formalin is effective in inhibiting the development of *Ascaris suum* eggs to the larval stage. It suggests that although formalin can maintain the structural integrity of the egg for diagnostic purposes, formalin may not support the complete development from the morula to the larval

stage. The likelihood that *Ascaris lumbricoides* eggs in formalin-preserved feces will develop from the morula stage to larvae is small because formalin, especially in higher concentrations, tends to inhibit egg development and prevent it from reaching the larval stage (Zhang et al., 2024).

However, in this study, the results showed that the addition of formalin at 10%, 15%, 20%, 25%, and 30% prevented the development of eggs from the morula stage onward, resulting in eggs containing larvae. It can be caused because *Ascaris lumbricoides* eggs have a strong and complex structure, which consists of three different layers, namely a sticky and notched outer layer that helps the egg stick to the surface and facilitates transmission; The Middle layer consists of chitin, and this layer provides structural integrity and protection against environmental stress; as well as the inner layer containing mucopolysaccharides and proteins to maintain the resistance of the egg and its ability to survive harsh conditions (Beyhan et al., 2016; Quilès et al., 2006).

The three-layer structure makes *Ascaris* eggs highly resistant to various environmental factors, including chemical treatments. For example, acetic acid must be at least 5% for 30 minutes to effectively kill the egg (Beyhan et al., 2016). The layers of chitin, mucopolysaccharides, and proteins in the eggshell are essential for its durability (Quilès et al., 2006).

This result is in line with previous research, namely that *Ascaris lumbricoides* worm eggs are still able to survive in a 10% formalin solution and are still able to develop into an infective form even though the 10% formalin is heated to temperatures of 60°C, 70°C, and 80°C and immediately added to faeces containing *Ascaris lumbricoides* eggs (Aryadnyani & Inderiati, 2018). In addition, in a controlled environment, such as in vitro culture with sulfuric acid, *Ascaris* eggs can develop from the morula stage to the larval stage within a few weeks (Cruz et al., 2012; Nisha et al., 2022).

However, in another study, it was found that *Ascaris lumbricoides* eggs with a morula in faeces treated with 10% formalin and heated to 80°C, 90°C, or 100°C for 10-30 minutes can prevent the eggs from developing into larvae (Suhu et al., 2020). The three-layered structure of *Ascaris lumbricoides* eggs is an important factor in their durability and resistance, making them a challenge to prevent transmission. Therefore, it is necessary to continue studying the layers of the egg wall and their biochemical properties to develop effective control measures.

4. Conclusion

This study demonstrates that formalin at concentrations of 10%, 15%, 20%, 25%, and 30% is unable to inhibit the development of *Ascaris lumbricoides* eggs from the morula stage to eggs containing infective larvae. The findings indicate that formalin has limitations in preventing the biological development of *Ascaris lumbricoides* eggs. Therefore, parasitological examination results need to consider the potential for changes in the egg stage during storage. The limitations of this study include the lack of evaluation of other environmental factors, such as temperature and more extended storage periods, and the absence of comparisons with alternative preservatives. Further research is recommended to explore combinations of other preservative concentrations, variations in storage conditions, and molecular or larval viability approaches to gain a more comprehensive understanding that supports the accuracy of laboratory-based parasitological diagnosis.

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Conflict of Interest

The authors declare no conflict of interest.

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