

# *The Detection of Toxoplasma gondii in Cat Feces Using the Microscopic and Loop-Mediated Isothermal Amplification (LAMP) Method at The Larangan Market Sidoarjo*

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## Abstract

*Toxoplasmosis is a zoonotic disease caused by Toxoplasma gondii, which has a significant impact on humans and animals, especially individuals with weakened immune systems. This study aimed to detect T. gondii in cat feces using the saturated NaCl microscopy method, and Loop-Mediated Isothermal Amplification (LAMP) method, as well as to the LAMP method and to evaluated the sensitivity of the LAMP method. Sampling was conducted using the accidental sampling technique over one month in August. The study employed a descriptive method by comparing result obtained through the microscopy and LAMP methods, analyzed using the SPSS version 23 kappa test. The detection result showed 4 positive samples and 5 negative samples out of 9 cat fecal samples from Larangan Market Sidoarjo, tested through NaCl flotation and DNA isolation using the resin method, followed by LAMP reaction with color change indicators. Sensitivity testing was performed by diluting the samples five times PBS to optimize DNA quality. The analysis showed perfect agreement between the two methods ( $k = 1.000$ ) with ( $p < 0,05$ ), indicating high significance in detecting T.gondii. Future research is recommended to expand the number and types of cat feces samples form various market locations to echange population representation and the validity of the research findings.*

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## INTRODUCTION

Toxoplasmosis is a zoonotic disease caused by the intracellular parasite *Toxoplasma gondii*. This disease has a significant impact on both human and animal health, particularly in individuals with weakened immune systems (Budi et al., 2022). In addition, toxoplasmosis also affects the health of cats as definitive hosts, which play a crucial role in the parasite's life cycle (Mose et al., 2020). The disease requires an interdisciplinary approach for its prevention, diagnosis, and control, making early and accurate detection of *T. gondii* infection in cats a vital step in protecting public and animal health (Aguirre et al., 2019).

Cats, as definitive hosts, play a primary role in the transmission of toxoplasmosis. This species is capable of shedding oocysts into the environment, where they can survive for extended periods and serve as a potential source of infection to humans and other animals (Rahman & Nur, 2022). This study aims to detect that outdoor cats are at a higher risk of *T. gondii* infection due to their behavior, including the consumption of raw meat and exposure to environments contaminated with *T. gondii* oocysts. These oocysts, excreted in cat feces, represent the most environmentally relevant form of the parasite for transmission, as the trophozoite stage is found only during active infection in host tissues and is not applicable to environmental sampling such as feces from cats in traditional markets (S. Al-Malki, 2021). The frequent interactions between humans and cats in public areas, including traditional markets, further increase the risk of toxoplasmosis transmission (Rahman & Nur, 2022).

According to data from the World Health Organization (WHO), the global prevalence of *T. gondii* infection in humans ranges from 25–30%, depending on geographic region and sanitation conditions (Torgerson & Mastroiacovo, 2013). In Indonesia, the seroprevalence of *T. gondii* is widespread among various human and animal populations, with infection rates ranging from 43–88% in humans and 35–75% in cats (Retmanasari et al., 2017). The presence of cats in densely populated environments such as Pasar

Larangan in Sidoarjo Regency indicates a potential hotspot for the spread of this parasite (Zakaria & Ardiansyah, 2020). The crowded conditions and frequent human-animal interaction in such markets may enhance the risk of oocyst dissemination through direct contact with cats or indirectly via contaminated surroundings (Brebes et al., 2017).

Currently, the detection of toxoplasmosis in cats still faces challenges using conventional methods such as serological tests and microscopic examinations, which suffer from limitations in sensitivity, specificity, and time efficiency. To improve diagnostic accuracy, the Loop-Mediated Isothermal Amplification (LAMP) method has emerged as an innovative alternative. This method enables rapid detection with high sensitivity and specificity and can be applied directly in field settings using simple equipment (Mirahmadi et al., 2020). This study aims to detect *T. gondii* in cat feces using the LAMP method and to assess its sensitivity in detecting *T. gondii*, thereby contributing to improved diagnostic strategies, particularly for cats as the primary vector (Augustine et al., 2020).

Through this research, it is expected that the risk of toxoplasmosis infection in humans and animals can be minimized, especially for vulnerable populations such as pregnant women and immunocompromised individuals, including those with HIV/AIDS, cancer, or organ transplants. The LAMP technology is anticipated to provide an effective solution for the development of more reliable and efficient *T. gondii* detection methods, thus supporting comprehensive efforts in toxoplasmosis control (Notomi et al., 2015).

## **METHOD**

This study employed a descriptive-exploratory design to identify the presence of *Toxoplasma gondii* in cat feces using the Loop-Mediated Isothermal Amplification (LAMP) method. The study received ethical clearance from Universitas Airlangga, Surabaya, with certification number: No.0849/HRECC.FODM/VII/2024. The study subjects included the entire population of cats found at the Larangan Market, Sidoarjo.

Fecal sample collection was conducted at the animal laboratory in the morning during August 2024, using an accidental sampling technique for one month. The samples were obtained from cat feces collected in labeled sample containers, stored in an icebox, and transported to the laboratory for examination. The research was carried out at the Molecular Biology and Histopathology Laboratory, Faculty of Medicine, Universitas Muhammadiyah Sidoarjo, during December 2024. The cats were brought from Larangan Market to the Animal Laboratory and kept for a full day to collect fresh feces the following morning for analysis.

*T. gondii* detection was performed using two methods: saturated NaCl flotation microscopy and the LAMP technique. In the microscopy method, 2 grams of cat feces were weighed and mixed with saturated NaCl solution up to  $\frac{3}{4}$  of the tube volume, then left to stand for 1 hour to allow oocysts to float to the surface. The surface layer was transferred to a cover glass and observed under a microscope at 400x magnification.

For the LAMP method, DNA was first isolated using the resin method, followed by LAMP amplification and a sensitivity test. Visualization of the results was conducted using a UV-Transilluminator. The LAMP reaction mixture included: 8  $\mu$ l DNA, 1.5  $\mu$ l BSM Taq, 3.5  $\mu$ l ddH<sub>2</sub>O, 12.5  $\mu$ l PCR mix, 1  $\mu$ l FIP primer (5'-CGCCTTTAGCACATCTGGTTCGAGATGCTCAAAGTCGACCGC-3'), 1  $\mu$ l BIP primer (5'-TATCGCAACGGAGTTCTTCCCAGGGCCTGATATTACGACGGAC-3'), 1  $\mu$ l F3 primer (5'-GGGAGCAAGAGTTGGGACTA-3'), and 1  $\mu$ l B3 primer (5'-CAGACAGCGAACAGAACAGAA-3').

The LAMP reaction was carried out at 60°C for 60 minutes, followed by heating to 80°C for 2 minutes to inactivate the polymerase. A positive result was indicated by a color change from purple to red, while no color change or a murky red indicated a negative result under the UV Transilluminator.

Sensitivity testing was performed by diluting the sample five times using PBS (Phosphate Buffered Saline). Each microtube was filled with 20  $\mu$ l of DNA and 20  $\mu$ l of PBS, and 20  $\mu$ l of the mixture was used to test LAMP sensitivity by observing sample turbidity visually under the UV Transilluminator.

Data were analyzed descriptively, and statistical analysis was conducted using SPSS version 23. A Kappa test was performed at a significance level of  $p < 0.05$  to assess the consistency between microscopy and LAMP method results.

## **RESULTS AND DISCUSSION**

Sample collection was conducted over a one-month period to ensure broader representation of the cat population at Larangan Market, Sidoarjo. Each sample was obtained from a different cat, allowing for variability in test results and minimizing the risk of duplicate sampling from the same individual. The

distribution of cat fecal samples collected in the Larangan Market area, Sidoarjo, is illustrated in Figure 1, which provides a graphical overview of the number of fecal samples collected per week.

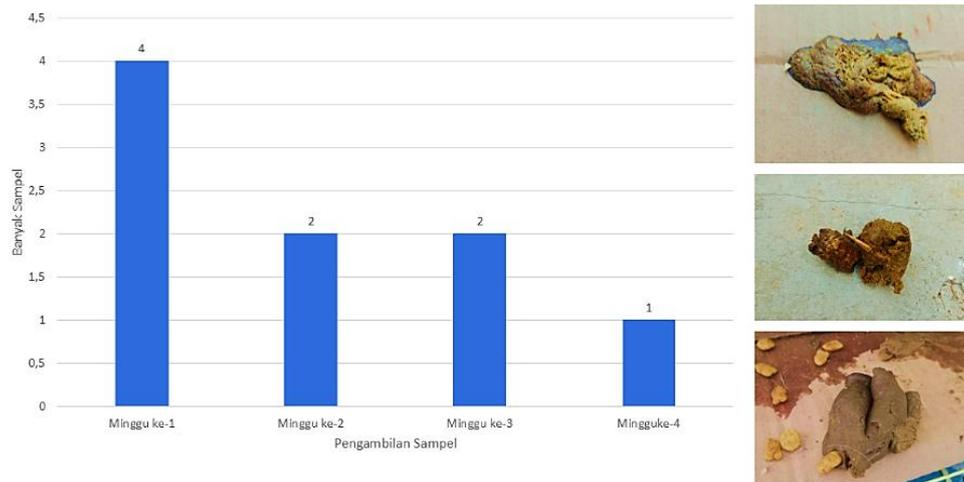
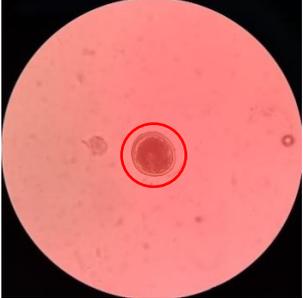


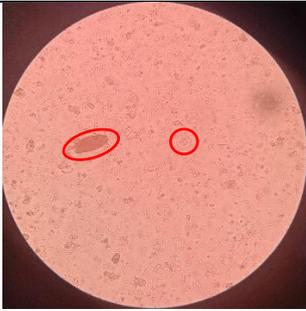
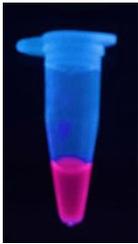
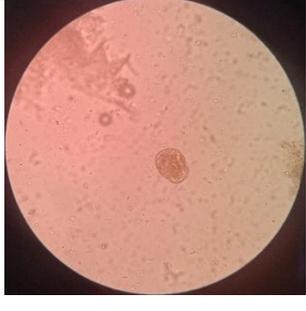
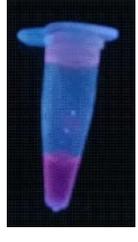
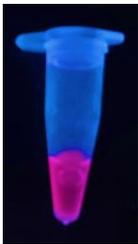
Figure 1. The Number of Cat Fecal Samples Collected from Week 1 to Week 4 at Larangan Market, Sidoarjo (left), and the Physical Condition of Cat Fecal Samples (right).

Figure 1 — The graph on the left illustrates the number of cat fecal samples obtained from Larangan Market, Sidoarjo, during Weeks 1 to 4 in the month of August. In Week 1, 4 samples were collected; in Week 2, 2 samples; in Week 3, 2 samples; and in Week 4, 1 sample. Among the four weeks, Week 1 yielded the highest number of fecal samples. Meanwhile, the image on the right shows the physical condition of the collected cat fecal samples, including their texture, color, and shape.

**Table 1.** Identification Results of *Toxoplasma gondii* in Cat Feces Using Microscopic and LAMP Methods

Sample	Microscopic Examination	LAMP Test	Description
Sample 1	 (-)	 (-)	<i>Toxocara cati</i>
Sample 2	 (+)	 (+)	<i>Toxoplasma gondii</i>

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Sample 3			<i>Toxoplasma gondii</i> & <i>Trichuris trichiura</i>
	(+) (+)	(+)	
Sample 4			Presence of Contaminants in the Sample
	(-)	(-)	
Sample 5			<i>Toxoplasma gondii</i>
	(+) (+)	(+)	
Sample 6			<i>Taenia sp.</i>
	(-)	(-)	

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Sample 7			<i>Trichuris trichiura</i>
	(-)	(-)	
Sample 8			<i>Toxoplasma gondii</i>
	(+)	(+)	
Sample 9			Presence of Contaminants in the Sample
	(-)	(-)	
Negative Control			Presence of Contaminants in the Sample
		(-)	

The results of the microscopic method using saturated NaCl solution at 40x magnification showed the presence of *T. gondii* oocysts in sample codes 2, 3, 5, and 8 out of a total of 9 samples. These oocysts appeared as round or oval structures with clear and thick walls. Additionally, several other types of parasites, aside from *T. gondii*, were found in the cat feces samples, including *Toxocara cati* in sample number 1, *Trichuris trichiura* in samples number 3 and 7, and *Taenia sp.* in sample number 7. Table 1 also shows the DNA results for *T. gondii* using the LAMP method on the cat feces samples, with 5 negative samples (samples 1, 4, 6, 7, and 9) and 4 positive samples (samples 2, 3, 5, and 8) out of a total of 9 samples. The test results were marked by a color change from purple to red in the positive reaction, whereas no color change indicated a negative reaction under the UV Transluminator.

**Table 2.** The number of *T. gondii* samples identified using the Microscopic Method and LAMP Method based on the Kappa Test

<i>T.gondii</i> Examination Results	Method		Kappa Test ( <i>p</i> -value)
	Saturated NaCl Flotation	LAMP	
Positive	4	4	
Negative	5	5	0,003
Total	9	9	

From Table 2, it can be seen that the percentage of *T. gondii* infection in cat feces at Pasar Larangan, Sidoarjo, using the microscopic method and LAMP method based on the Kappa test showed a significant result ( $p < 0.05$ ) with a total of 9 samples consisting of 4 positive samples and 5 negative samples.

Figure 2 shows the results of the sensitivity test of the LAMP method, conducted using the serial dilution technique. The results indicate that the lowest detectable concentration of target DNA was at the 3x dilution, as the sample at the 4x dilution already exhibited a color change.

*Toxoplasma gondii* (*T. gondii*) is a parasitic protozoan that can cause toxoplasmosis, a disease with the potential to become a serious health issue in both humans and animals. One of the molecular methods used to detect *T. gondii* is Loop-Mediated Isothermal Amplification (LAMP). The LAMP method is known as an efficient, simple, and sensitive genetic amplification technique, making it highly suitable for use in both clinical and field testing (Sheng et al., 2023).

The microscopic method is performed to directly detect the presence of *T. gondii* oocysts in cat feces. As a gold standard, this method is crucial for confirming the results obtained from molecular methods such as LAMP. The combination of these two methods can provide more comprehensive and accurate results, thereby enhancing the reliability of *T. gondii* diagnosis. The first step in the saturated NaCl flotation microscopic method involves weighing 2 grams of cat feces, followed by the addition of saturated NaCl solution. Due to its ability to increase the solution's density, the *T. gondii* oocysts, which have lower density, will float to the surface (Zakaria & Ardiansyah, 2020).

The initial stage in detecting *T. gondii* using molecular methods is DNA isolation. Before DNA isolation, the sample—cat feces—must be prepared to separate *T. gondii* from unwanted substances such as debris or dust. This preparation involves saturated NaCl solution, allowing *T. gondii* to float at the top. The fecal sample is then centrifuged, causing *T. gondii* to settle at the bottom of the tube, ready for further analysis (Jayanti & Mushlih, 2021). The quality of the DNA obtained is heavily influenced by the DNA isolation stage. DNA isolation aims to separate DNA from other components such as proteins, carbohydrates, and lipids. In this study, a resin-based isolation method was used, employing ddH<sub>2</sub>O (deionized water) and Instagen as supporting reagents (Setiaputri et al., 2020).

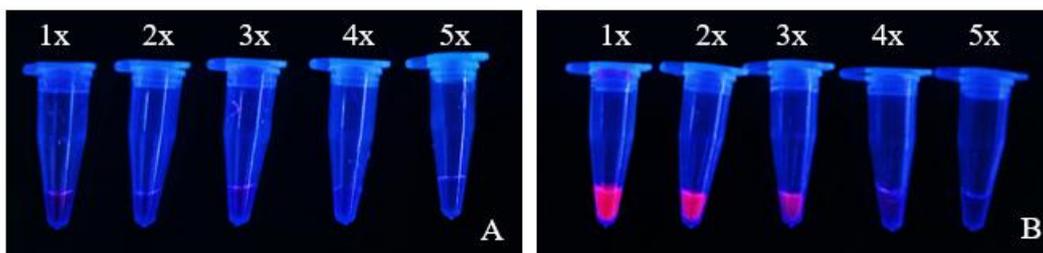


Figure 2. Sensitivity test results of the LAMP method on *T. gondii* samples using dilution with PBS (Phosphate Buffer Saline) solution: Sensitivity test result of a negative sample (A), Sensitivity test result of a positive sample (B).

In this study, detection of *T. gondii* was performed using the saturated NaCl microscopic method and the LAMP method on a total of 9 cat fecal samples collected from Pasar Larangan, Sidoarjo, comprising 4 positive and 5 negative samples. Prior to detection, the samples were isolated using the resin method. Following detection using the LAMP method, a dilution process using PBS (Phosphate Buffered Saline) was

conducted five times. This dilution aimed to improve sample quality by removing contaminants. The use of PBS also enhances the isolation of parasite DNA for use in the LAMP method.

Using saturated NaCl and 40x10 magnification in the microscopic method, several types of parasites other than *T. gondii* were found in the cat fecal samples, including *Toxocara cati* in sample no. 1, *Trichuris trichiura* in samples no. 3 and 7, and *Taenia* sp. in sample no. 7. The presence of these parasites indicates that cats, in addition to being potential hosts for *T. gondii*, also serve as reservoirs for other parasites of clinical and zoonotic significance.

The LAMP method offers advantages in sensitivity, even for samples with low DNA concentrations. In this study, successful detection was indicated by a color change at each level of sample dilution. LAMP commonly uses colorimetric indicators based on pH or metal ions such as Hydroxy Naphthol Blue (HNB), where color change signifies successful DNA amplification. This visible change is significant and easily observed with the naked eye, making LAMP a highly practical and reliable method (Park, 2022).

The successful detection of four *T. gondii*-positive samples using the LAMP method demonstrates its ability to specifically detect *T. gondii* DNA. Sensitivity testing was performed repeatedly on both positive and negative samples, up to five times. This high sensitivity is a key advantage of the LAMP method over other molecular methods such as conventional PCR. Additionally, LAMP does not require complex thermal cycling, as amplification occurs isothermally (at a constant temperature), making it faster, cost-effective, and applicable in basic laboratory settings. Conversely, for the five negative samples, no color change was observed during testing, indicating the LAMP method's high specificity in detecting the target DNA. This also suggests that LAMP can effectively differentiate between *T. gondii*-positive and -negative samples, reducing the likelihood of false positives or negatives.

The findings are consistent with the study by Zakaria and Ardiansyah (2020), which used cat fecal samples from three markets in Sidoarjo Regency: Pasar Larangan, Pasar Suko, and Pasar Sukodono. In that study, 3 out of 8 cat fecal samples from Pasar Larangan were positive for *T. gondii*, indicating the potential for *T. gondii* transmission among stray cats in the area.

Based on statistical analysis using SPSS and the Kappa test, a value of  $\kappa = 1.000$  indicated perfect agreement between the saturated NaCl flotation method and the LAMP method for detecting *T. gondii* in cat fecal samples. This perfect agreement implies that both methods yielded identical results for the tested samples. Moreover, a significance value of  $p < 0.05$  confirms that the results are statistically significant, indicating that both methods can be used reliably and consistently in detecting *T. gondii*.

Dilution is a common technique in molecular sensitivity testing, including for the LAMP method. This technique aims to determine sensitivity levels by indicating the lowest concentration of target DNA that can still be detected. In this study, serial dilution using PBS solution was conducted to produce varying DNA concentrations. This approach allowed researchers to evaluate the effectiveness of the LAMP method at different DNA concentrations and to determine the minimum concentration that still produced a positive amplification result (Rahmah et al., 2024).

The LAMP sensitivity test results demonstrated the method's ability to detect the target DNA down to a certain concentration. Serial dilution was used to determine the detection limit—the lowest DNA concentration that can still be accurately identified using the LAMP method (Rahmah et al., 2024). Based on the results, the minimum detectable concentration was at the 3x dilution, indicating that at this level, LAMP could still positively detect the target DNA. However, at the 4x dilution, the sample exhibited a color change suggesting that amplification did not occur or detection failed at this lower concentration.

Determining this detection threshold is important because it reflects the reliability of the LAMP method in detecting DNA at low concentrations. This is particularly relevant in diagnostic amplification such as *T. gondii* detection, where samples often contain very small amounts of DNA. These results demonstrate that the LAMP method has sufficiently high sensitivity to detect low-concentration target DNA.

Given its high sensitivity and specificity, the LAMP method offers an effective solution for detecting *T. gondii* in cat feces, particularly in resource-limited laboratory settings. The use of LAMP can improve diagnostic accuracy for toxoplasmosis in animals and indirectly assist in controlling disease transmission to humans (zoonosis). The observable color change also makes this method very user-friendly for healthcare workers or personnel with minimal experience, further expanding its application in public and animal health surveillance (Augustine et al., 2020).

The correlation between the microscopic and LAMP methods in this study suggests that both can be used complementarily. Microscopy can serve as an initial screening tool for identifying oocysts, while LAMP provides more accurate molecular confirmation. If both methods yield consistent results (positive or

negative), the validity of the findings is considered high. However, in cases where results do not match (e.g., negative by microscopy but positive by LAMP), it may indicate a low amount of *T. gondii* DNA or oocyst morphology not visible under the microscope.

In epidemiological and environmental detection contexts, the simultaneous use of both methods is highly recommended to increase detection sensitivity and reduce the risk of diagnostic errors. Thus, a combined approach using microscopy and molecular methods such as LAMP offers greater potential for obtaining a comprehensive overview of *T. gondii* distribution in the environment. The inclusion of microscopy as a gold standard remains essential. In both research and clinical applications, microscopy and molecular methods provide complementary diagnostic approaches—microscopy offers visual confirmation, while LAMP delivers rapid and sensitive molecular detection.

## CONCLUSION

Based on the results of the study, it can be concluded that the detection of *T. gondii* in cat feces using the saturated NaCl flotation microscopy method and the LAMP method yielded 4 positive samples and 5 negative samples out of a total of 9 samples. Both the saturated NaCl flotation method and the LAMP method demonstrated a high level of reliability, indicating that either method can be used independently or in combination for the diagnostic examination of toxoplasmosis. However, the advantages of the LAMP method in terms of speed and sensitivity provide a superior edge over the saturated NaCl flotation method.

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