

ORIGINAL ARTICLE

Identification of *Toxoplasma gondii* oocysts in feces of purebred cats in a cattery using flotation method

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ABSTRACT

Toxoplasmosis is a zoonotic disease caused by the protozoan *Toxoplasma gondii*, with cats serving as the definitive host that shedding oocysts through their feces. Cattery environments have the potential to become sources of oocyst transmission if sanitation and management systems are not properly maintained. This study aimed to identify the presence of *Toxoplasma gondii* oocysts in the feces of purebred cats at a cattery using the flotation method. This study used a descriptive design. Fecal samples were collected from a single cattery on two occasions, one week apart, utilizing a stratified random sampling method. During each collection, seven fecal samples from purebred cats were randomly collected from the available litter boxes, which served as the strata, and each sample was used to prepare three slides. Examination was performed microscopically using the flotation method with a saturated NaCl solution. A total of 42 slides were examined. The results showed that all slides prepared from purebred cat fecal samples were negative for *Toxoplasma gondii* oocysts in both the first and second sampling periods, resulting in a 100% negative rate. These results indicate that during the study period, no shedding of *Toxoplasma gondii* oocysts was detected by microscopic examination of fecal samples from the purebred cats at the cattery. This study concludes that no *Toxoplasma gondii* oocysts were found in the feces of purebred cats at the studied cattery, while taking into account the limitations of the flotation method and sample collection from a shared litter box.

Keyword: cattery, feces, flotation method, purebred cats, *Toxoplasma gondii*

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INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan that causes toxoplasmosis, a zoonotic disease capable of infecting humans and various animal species, with cats serving as the definitive hosts in the parasite's life cycle through the excretion of oocysts in feces.¹ While the infection is often asymptomatic, toxoplasmosis poses a severe threat to immunocompromised individuals and pregnant women, as it can cause congenital abnormalities, abortion, or central nervous system complications.² In immunocompromised patients, particularly those with Acquired Immunodeficiency Syndrome (AIDS), reactivation of latent infection may lead to toxoplasmic encephalitis and severe neurological disorders.³ Oocysts excreted in cat feces can persist for extended periods in the environment as a source of transmission and require 1-5 days to sporulate before becoming infective to humans and other intermediate hosts.^{4,5} In general, *Toxoplasma gondii* can be transmitted to humans through three primary routes: the consumption of food or water contaminated with tissue cysts containing bradyzoites from undercooked meat, direct contact with an environment contaminated with sporulated oocysts from cat feces, and vertical transmission from an infected mother to her fetus. Because cats are the only definitive hosts capable of shedding oocysts into the environment, their role is exceptionally important in the epidemiological chain.

According to the World Health Organization (WHO), *Toxoplasma gondii* infection is distributed globally. In Indonesia, the prevalence of this infection varies significantly across regions, with the highest prevalence recorded at 58% in North Sulawesi, followed by 51% in West Java and 31% in South Kalimantan.⁶ A cattery environment, characterized by high population density and shared litter boxes, has the potential to increase the risk of *Toxoplasma gondii* oocyst transmission if

management and hygiene systems are not properly maintained.^{7,8} Nevertheless, research regarding the identification of *Toxoplasma gondii* oocysts in the feces of purebred cats in cattery environments in Indonesia remains limited, as previous studies have focused primarily on feral cats or household pets.⁹ This study focuses on an officially registered World Cat Federation (WCF) cattery located in Bandung, Indonesia, where purebred cats may be exposed to risk factors such as feeding raw meat, which may increase the risk of infection through the ingestion of tissue cysts present in intermediate hosts.¹⁰

Establishing a clinical diagnosis of toxoplasmosis is often challenging due to non-specific symptoms, making microscopic fecal screening an essential initial step. While the direct smear method is frequently used, it has a major drawback because it uses a very small sample size, potentially leading to false-negative results. Therefore, concentration methods, specifically the simple flotation method, are frequently preferred. The flotation method is commonly used in parasitology to detect *Toxoplasma gondii* oocysts because it concentrates parasitic material from a larger volume of feces, offering a practical, cost-effective, and potentially more suitable diagnostic approach compared to direct smears, although its sensitivity may be influenced by the number of oocysts present in the sample.¹¹ Based on this background, the present study utilized a descriptive design to identify the presence of *Toxoplasma gondii* oocysts in the feces of purebred cats at the studied cattery using the flotation method.

METHODS AND SUBJECTS

This descriptive study aimed to identify the presence of *Toxoplasma gondii* oocysts in the feces of purebred cats within a cattery environment.² The study was conducted at a single officially registered cattery located in Bandung, Indonesia, while laboratory examinations were performed at the Parasitology Laboratory

of the Faculty of Medicine, Universitas Jenderal Achmad Yani. During the study period, the cattery housed 10 purebred cats that were included in the study. The population was dynamic due to the birth of one kitten during the study period, but the newborn kitten was not included in the study population. Samples were collected twice at one-week intervals to account for the potential *Toxoplasma gondii* oocyst shedding period, which generally occurs approximately 10–14 days after primary infection.⁵

The study subjects were 10 adult purebred cats aged ≥ 1 year kept at the cattery that showed no clinical signs of illness and were not undergoing any medical treatments, while the study object was the detection of *Toxoplasma gondii* oocysts present in cat feces. Fecal samples were collected using the total sampling method from all available litter boxes at the cattery, totaling seven units, with each litter box treated as a single stratum.⁶ The seven available litter boxes were shared by approximately 1–5 cats during the study period, depending on the location and accessibility of each litter box. From each litter box, approximately 5 grams of fresh feces were randomly collected from the surface using a sterile spatula within 24 hours. The use of bentonite clumping sand in the cattery facilitated the separation of feces, ensuring that the samples were free from sand or urine. The freshly collected samples were placed in sterile containers and immediately transported to the laboratory for examination. Three examination slides were prepared from each sample. Thus, 21 slides were obtained at each sampling time and a total of 42 slides were obtained throughout the study. During the sampling and handling process, strict biosafety measures were applied, including the use of personal protective equipment (PPE).

A fecal examination was performed using the flotation method, which is a standard parasitological technique used to detect the presence of *Toxoplasma gondii*

oocysts.¹⁰ In the laboratory, exactly 2 grams of the collected fecal samples were weighed and homogenized with distilled water (aquades). The mixture was filtered using a tea strainer to remove coarse particles and initially centrifuged at 1200 rpm for 3 minutes. After the supernatant was discarded, the sediment was mixed with a saturated NaCl solution up to approximately three-quarters of the tube's volume. The saturated NaCl solution used as the flotation medium had an approximate specific gravity of 1.18–1.20. The mixture was then centrifuged again at 1200 rpm for 3 minutes. The principle of the flotation method relies on the difference in specific gravity between the oocysts and the flotation solution, allowing the oocysts to float to the surface.¹² The tube was then placed upright, and additional saturated NaCl solution was carefully added using a Pasteur pipette until a convex meniscus was formed. After standing for 1–2 minutes, the floating materials were collected with a cover slip.¹³ The specimen was then examined using a light microscope at 40 $\times$ magnification to identify the presence of *Toxoplasma gondii* oocysts based on their morphological characteristics.

Data analysis was performed using a descriptive quantitative approach based on the results of examination of fecal samples collected from the litter boxes. The statistical unit of analysis was the litter box stratum rather than individual cats because fecal samples were collected from shared litter boxes. A sample was classified as positive if oocysts were detected and negative if no oocysts were found.¹⁴ Data are presented in tables and as percentages to illustrate the presence of *Toxoplasma gondii* oocysts in the examined fecal samples. This study received ethical approval from the Research Ethics Committee of the Faculty of Medicine, Universitas Jenderal Achmad Yani, as documented in letter **No. 004/UH1.01/2026.**

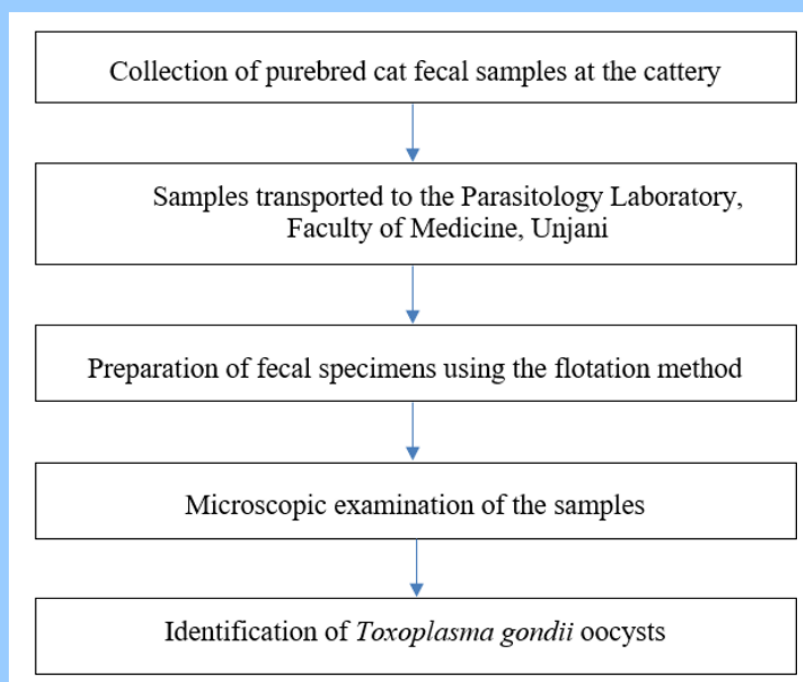


Figure 1. Flowchart

RESULTS AND DISCUSSION

Results

This study aimed to identify the presence of *Toxoplasma gondii* oocysts in the feces of purebred cats at a cattery using the flotation method. The study was conducted in a cattery with a dynamic population due to the birth of one kitten during the study period. Fecal samples were collected twice at one-week intervals to account for the parasite's specific oocyst shedding period. At each sampling time, seven fecal samples were collected from seven litter boxes, and each fecal sample was used to prepare three microscopic slides, resulting in 21 slides per sampling period and a total of 42 slides

examined throughout the study.¹⁵

The laboratory identification involved a comprehensive microscopic examination at 40× magnification. The observation specifically looked for the morphological characteristics of *Toxoplasma gondii* oocysts, which are typically spherical or oval and measure approximately 10 to 12 µm in diameter.³ However, the microscopic examination results showed that no *Toxoplasma gondii* oocysts were detected in any of the examined fecal samples from either the first or second sampling period. All seven fecal samples collected from the litter boxes tested negative results for oocyst detection during both sampling periods.

Table 1. Summary of *Toxoplasma gondii* Oocyst Examination Results from Fecal Samples Collected at the Cattery

Sampling Period	Total of Samples	Positive Samples	Negative Samples	Detection Rate
First Week	21	0	21	0%
Second Week	21	0	21	0%
Total	42	0	42	0%

The summary of examination results from both sampling periods is presented in Table 1. A total of 14 fecal samples collected

from seven litter boxes over two sampling periods were examined, and all samples tested negative for *Toxoplasma gondii* oocyst.

Discussion

The results of this study indicate that *Toxoplasma gondii* oocysts were not found in any of the fecal samples from purebred cats at the studied cattery. This finding suggests that during the sampling period, the purebred cats at that cattery were not detected as shedding oocyst or had a very low risk of environmental contamination. Based on direct observation, the cattery environment was well-maintained and managed. The viability and sporulation process of *Toxoplasma gondii* oocysts are highly dependent on environmental factors such as temperature and humidity. Theoretically, newly excreted oocysts are not immediately infective; they require 1-5 days to sporulate at approximately 24°C, and can survive for 14 to 18 days in environments with 44–80% humidity.^{3,16} In this study, fecal samples were collected within 24 hours of excretion. This routine cleaning management prevents any potential oocysts from sporulating, thereby potentially breaking the chain of transmission. Furthermore, a clean, dry cattery environment with good air circulation actively inhibits oocyst viability, reducing the chances of environmental contamination.

These negative findings contrast with several other studies in Indonesia that reported the presence of *Toxoplasma gondii* in cat feces and a high prevalence of toxoplasmosis among human populations. For instance, a previous study in Surabaya demonstrated that approximately 64% of women of reproductive age were infected with toxoplasmosis, and 30% of these infected individuals owned more than two cats at home.¹⁰ This highlights the potential risk associated with high-density cat populations, such as those in a cattery, particularly when adequate hygiene practices are not maintained. The discrepancy between the theoretical risk and the negative findings in this study may be attributed to differences in rearing systems, environmental sanitation, and limited contact with feral cats or external sources of infection in professionally

managed catteries.

Another critical aspect influencing these results is the combination of the cats' diet and age factors. Observations revealed that the cattery provided a mixed diet of commercial dry food and raw meat. Breeders frequently engage in this practice, believing it helps build the cat's anatomy and body shape to meet international feline organization standards.⁵ While feeding raw meat is a known risk factor because the muscle tissues of intermediate hosts may contain infectious bradyzoites (tissue cysts), the absence of oocysts indicates that the raw meat provided may have been properly frozen or processed to inactivate the tissue cysts, or that the cats were not actively shedding oocysts during the sampling period.

Additionally, all examined fecal samples originated from adult cats. Age plays a pivotal role in the epidemiology of toxoplasmosis. Kittens under one year of age are considerably more susceptible to infection because they have not developed specific antibodies. During a primary infection, kittens can shed millions of oocysts daily, making them a potentially important source of environmental contamination.³ Conversely, adult cats generally possess a more mature immune system capable of controlling the infection, thereby reducing the likelihood of *Toxoplasma gondii* oocyst shedding.

Furthermore, it is important to note that the clinical presentation of toxoplasmosis in cats can be highly deceptive. While acute infections may present with non-specific clinical signs such as lethargy, anorexia, fever, vomiting, diarrhea, or respiratory distress, the vast majority of feline infections remain entirely asymptomatic.³ Consequently, relying solely on the physical appearance or apparent health status of the purebred cats is insufficient to rule out the risk of active oocyst shedding. This finding underscores the importance of routine laboratory screening, even in professionally managed catteries where cats appear clinically healthy.

The absence of *Toxoplasma gondii* oocysts is also closely related to the parasite's specific life cycle (enteric cycle) and the limited duration of oocysts shedding in cats. The excretion of *Toxoplasma gondii* oocysts generally occurs for a relatively short, limited period, approximately 10 -14 days following a primary infection.³ After this period, cats generally do not continue to shed, although occasional shedding following reinfection has been reported. Therefore, even if a purebred cat in the cattery had been previously infected with *Toxoplasma gondii*, oocysts may not have been present in the feces at time of sampling. This indicates that the timing of sample collection is an important consideration, and a single cross-sectional fecal examination has temporal limitations in detecting the true infection status.

In terms of methodology, the simple flotation method was utilized due to its efficiency, cost-effectiveness, and ease of application. The principle relies on the specific gravity difference between the oocysts and the saturated NaCl solution. Saturated NaCl was specifically chosen because its specific gravity is sufficient to float the small *Toxoplasma gondii* oocysts without causing morphological deformation. Previous studies have reported that the simple flotation technique yields a higher sensitivity (25%) compared to the sedimentation method (20%), making it the heavily preferred choice for oocyst screening.¹⁷ However, its sensitivity remains relatively low when the number of oocysts present in a sample is low. While molecular (PCR) and serological methods, such as detecting IgG/IgM antibodies, may provide greater sensitivity for determining individual infection status, microscopic examination via flotation remains highly reliable as an essential initial screening tool, allowing for the direct visualization of intact, sporulated oocysts directly from environmental fecal samples.^{18,19}

The negative findings in this cattery may be related to the preventive practices implemented at the facility, such as the immediate removal of feces within 24 hours,

providing a combination of commercial and raw meat diets, and restricting access to feral, may help reduce opportunities for parasite transmission. The integration of strict environmental hygiene and proper sanitary handling of feline feces is an important measure for reducing the risk of zoonotic transmission.²⁰ According to veterinary guidelines, prevention can be further optimized through regular cleaning of enclosures with antiseptics and ensuring routine veterinary health checks at least once a month. In the event of a confirmed toxoplasmosis case, appropriate treatment regimens involving clindamycin (10–12 mg/kg administered orally) have proven effective in actively inhibiting the shedding of *Toxoplasma gondii* oocysts in cats.⁵ The integration of appropriate health and hygiene protocols in catteries may help reduce potential zoonotic risks to breeders and the public.

The implication of this study is that the implementation of excellent hygiene management in catteries, including regular litter box cleaning and appropriate feed management, effectively minimizes the risk of *Toxoplasma gondii* oocyst transmission to the environment. However, the limitations of this study include in its descriptive design, small sample size from a single cattery, and reliance on shared litter boxes for sample collection. The shared litter box approach made it difficult to attribute fecal samples to individual cats, affecting the exact interpretation of individual prevalence. Therefore, further research is recommended to combine multiple diagnostic methods, conduct individual sampling, and include more cattery locations to obtain a more comprehensive epidemiological picture.

CONCLUSION

Based on the results of this study, it can be concluded that no *Toxoplasma gondii* oocysts were found in the feces of purebred cats at the studied cattery using the flotation method; thus, during the sampling period, no oocysts shedding was detected in the fecal samples examined.

These findings indicate that a breeding system for purebred cats in a cattery, combined with excellent hygiene management, has the potential to significantly reduce the risk of toxoplasmosis transmission via feces. As a recommendation, future studies should consider using a combination of testing methods, including serological or molecular techniques, as well as comparing different microscopic methods like Sheather's sugar flotation. Furthermore, expanding the study locations to include various catteries and conducting individual fecal sampling from each cat over a longitudinal period will provide a more comprehensive and specific epidemiological picture of *Toxoplasma gondii* infection and oocyst shedding in purebred cats.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest in the writing of this scientific article.

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