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Toxoplasma Oocyst Contamination on Water Sources of Goat-Sheep Farming in Sidoarjo District

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ABSTRACT

Toxoplasmosis is one of the zoonotic diseases caused by the protozoa *Toxoplasma gondii* (*T. gondii*). The disease infects warm-blooded animals including humans. The purpose of this study was to determine the contamination of Toxoplasma oocysts in water sources in goat-sheep farms in Sidoarjo District. A total of 63 water samples were used for the study. Water samples were taken from 5 sub-districts that have many goat-sheep farms, each sub-district has 2 villages, with each village having 6-7 samples. The examination used the floating method and was examined under a microscope with a magnification of 400x. The results of the examination found 21 samples of *T.gondii* oocysts or 33.33% (21/63). The morphology of *T.gondii* oocysts is round, ovoid or ellipsoid with sizes varying between 8.02 - 12.72 µm. Preventive measures are needed by treating water before giving it to livestock.

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INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan that is zoonotic and infects about one-third of the world's human population. *T. gondii* infection can be transmitted through several routes to different hosts. Water or food contaminated with oocysts, undercooked meat, are the main sources of *T. gondii* infection in humans (Dubey, 2021). In Indonesia, the seroprevalence of toxoplasmosis in humans is 58.13% (Perdana, 2024). Sheep and goats are important sources of infection for humans, representing an important role in public health, because consumption of infected milk or meat can facilitate zoonotic transmission. In addition, toxoplasmosis is an important cause of neonatal death in sheep and goats, resulting in reproductive and economic losses (Belluco *et al.*, 2016; Fatmawati *et al.*, 2022). Based on a review by Fatmawati *et al.* stated that sheep milk samples positive for *T. gondii* DNA in Bahia were 10.5% and in Brazil 12.04%. Detection of *T. gondii* DNA in 625 sheep and goat milk samples in Iran with a prevalence of 3.04%, in sheep milk 4.63% and goat milk 1.07%. Molecular analysis of infected dairy goat milk in Italy showed that 20.6% of milk samples contained *T. gondii* DNA. In the Slovak Republic, the prevalence was 32.56% in goat milk. The percentage of *T. gondii* found in milk with values between 3.7%, 4.63%, and 7.8%. The results of serological tests using ELISA showed relatively high results of 90%, 38.9%, and 31.2% (Fatmawati *et al.*, 2022).

Toxoplasmosis has a socio-economic impact in the veterinary world, namely high treatment costs, decreased productivity, congenital abnormalities, abortion, and premature death (OIE, 2017). Toxoplasmosis is one of the important zoonotic diseases in the world because it causes birth defects of 2-8 million babies by the year (Smith *et al.*, 2021). Felidae, especially cats, are the definitive hosts of *T. gondii* (Dubey, 2021). Oocysts in feces are intermediaries for the transmission of the *T. gondii* parasite, where water is a good medium for the existence of oocysts from *T. gondii*. Oocysts that can live in water are sporulated oocysts. These sporulated oocysts can live and continue to contaminate soil, water and food (Shapiro *et al.*, 2019). Research related to toxoplasmosis in water sources on goat and sheep farms, especially in Sidoarjo Regency, East Java has not been carried out. Water sources are an important factor in the transmission of toxoplasmosis in animals and humans. Based on Purwantoro's research, the incidence of toxoplasmosis is related to work factors, the use of personal protective equipment, the habit of washing hands after contact with goats, the presence of cats, the presence of cat feces, the type of water source, the distance between the feed source and the river/drain, and the goat breed (Purwantoro, 2020). This study aims to determine the presence of *T. gondii* oocyst contamination in drinking water sources on goat and sheep farms in Sidoarjo Regency, East Java using a microscopic method.

MATERIALS AND METHODS

The study was conducted in Sidoarjo Regency with samples taken from water sources used as drinking for goats and sheep. The determination of sampling points was selected from 5 sub-districts with many goat and sheep farms and based on input from the relevant agencies. The number of samples was 63 water sources from 5 sub-districts with 2 villages each. Each village took between 6-7 samples with a volume of approximately 800 mL. Samples were left for at least 24 hours, so that they settle. The upper part was sucked and discarded with the help of a water hose, leaving approximately 50 mL of the lower part. The sediment was collected and centrifuged at a speed of 2500 rpm for 5 minutes. The supernatant was discarded, the sediment/pellet was dissolved with 200 µL of distilled water. The dissolved sediment was used for microscopic testing using the floatation method. Centrifuge tube (15 mL) containing dissolved sediment, the concentration results were then floated by adding saturated sucrose solution up to 1 mL from the mouth of the centrifuge tube. Centrifugation was carried out at 2500 rpm for 5 minutes, then saturated sucrose solution was added until a convex surface was formed. The mouth of the tube was covered with a cover glass, and left for 5 minutes. The cover glass was removed and examined using a Trinoculer Eclipse E200 microscope, 400X magnification. Positive results based on Sheng *et al*. (2023), if oocysts measuring 8- 12 µM were found, with a round, spherical shape, containing 2 sporocysts. The results were documented with a Nikon DS-Fi3 camera (Y-IDT Japan). Data are presented descriptively and in the form of Figures and Tables.

RESULTS AND DISCUSSION

The results of microscopic examination/ floatation test found 21 *T. gondii* oocysts (33.33%). The image of *T. gondii* oocysts can be seen in Figure 1 and the overall data can be seen in Table 1.

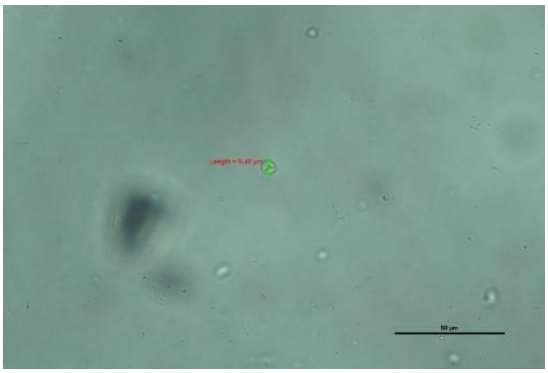

Figure 1. Morphology of *T. gondii* oocysts on drinking water sources goat-sheep farms

Table1. Results of Microscopic Examination/ Floating Method of *T. gondii* Oocyst Contamination on drinking Water Sources on Goat-Sheep Farms in Sidoarjo District.

Sub-District/Village	Total	Positive	%	Negative	%
BUDURAN					
Sawohan	7	2	21	5	79
Prasung	7	1		6	
SIDOARJO					
Sarirogo	6	3	41	3	59
Sumput	6	2		4	
CANDI					
Sepande	6	3	41	3	59
Kebonsari	6	2		4	
WONOAYU					
Becirongengor	6	2	30.7	4	69.3
Lambangan	7	2		5	
JABON					
Keboguyang	6	3	33.2	3	66.8
Kedungcangkring	6	1		5	
Total	**63**	**21**	**33.3**	**42**	**66.7**

Table 2. Results of Oocyst Measurements on Water Samples in Sidoarjo District.

Sub District	Village	Oocyst Size (µm)
Candi	Kebonsari	-
Candi	Kebonsari	-
Candi	Kebonsari	-
Candi	Kebonsari	8.48 ; 10.33
Candi	Kebonsari	-
Candi	Kebonsari	8.5; 9.23
Candi	Sepande	8.36
Candi	Sepande	11.29
Candi	Sepande	-
Candi	Sepande	-
Candi	Sepande	12.72 ; 8.97 ; 8.55
Candi	Sepande	-
Jabon	Keboguyang	-
Jabon	Keboguyang	-
Jabon	Keboguyang	9.43
Jabon	Keboguyang	8.9
Jabon	Keboguyang	-
Jabon	Keboguyang	8.7
Jabon	Kedung Cangkring	-
Jabon	Kedung Cangkring	9.13 ; 8.79
Jabon	Kedung Cangkring	-
Jabon	Kedung Cangkring	-
Jabon	Kedung Cangkring	-
Jabon	Kedung Cangkring	-
Sidoarjo	Sumput	-
Sidoarjo	Sumput	10.03
Sidoarjo	Sumput	-
Sidoarjo	Sumput	8.24 ; 8.03
Sidoarjo	Sumput	8.02 ; 8.27 ; 11.97 ; 10.16
Sidoarjo	Sumput	-
Sidoarjo	Sarirogo	-
Sidoarjo	Sarirogo	10.19
Sidoarjo	Sarirogo	11.43
Sidoarjo	Sarirogo	-
Sidoarjo	Sarirogo	-
Sidoarjo	Sarirogo	-
Buduran	Sawohan	-
Buduran	Sawohan	-
Buduran	Sawohan	8.45
Buduran	Sawohan	-
Buduran	Sawohan	-
Buduran	Sawohan	8.44
Buduran	Sawohan	-
Buduran	Prasung	8.44
Buduran	Prasung	-
Buduran	Prasung	-
Buduran	Prasung	-
Buduran	Prasung	-
Buduran	Prasung	-
Buduran	Prasung	-
Wonoayu	Becirongengor	10.64
Wonoayu	Becirongengor	-
Wonoayu	Becirongengor	-
Wonoayu	Becirongengor	-
Wonoayu	Becirongengor	-
Wonoayu	Becirongengor	8.85
Wonoayu	Lambangan	-
Wonoayu	Lambangan	9.96
Wonoayu	Lambangan	9.81; 8.52; 10.21
Wonoayu	Lambangan	-
Wonoayu	Lambangan	-
Wonoayu	Lambangan	-
Wonoayu	Lambangan	-
Total (%)		**21 (33.3%)**
Mean ± SD		**9.40 ± 1.2 µm**

The morphological form of *T.gondii* oocysts is round, ovoid or ellipsoid with sizes varying between 8.02 – 12.72 µm (Mean ± SD: 9.40 ± 1.2 µm) (Table 2). This size is in accordance with Sheng *et.al*. who reported the size of *T.gondii* oocysts between 8-12 µm (Sheng *el al.*, 2023), and 9-15 µm (Wana *et al.*, 2020).

The size of these oocysts is very small, so further examination with molecular tests is needed. In addition to molecular tests, biological tests need to be carried out to determine the infectivity of the oocysts found. Samples containing oocysts are inoculated into mice. It is expected that in the body of the mice, *T. gondii* will develop, so that several stages will be obtained, namely the trophozoite stage (in intraperitoneal fluid) or cysts (Berrouch *et al.*, 2023) containing bradyzoites (brain tissue). Biological tests require a minimum of 6-8 weeks to obtain the results of the development of the inoculated *T. gondii*.

The results of the study of *T. gondii* contamination in drinking water sources on goat-sheep farms in Sidoarjo Regency were higher than the *T. gondii* contamination in water sources reported by several country researchers. In Morocco, *Toxoplasma* contamination in water sources was 17.3% and combined contamination of *Toxoplasma* with *Giardia* was 16.3% (Berrouch *et al.*, 2023). Toxoplasmosis has been reported to be transmitted through drinking water from 1960 to 2020, around 34 acute toxoplasmosis outbreaks were reported worldwide, of which 21% were waterborne (Dubey, 2021). Outbreaks in water sources in Brazil were 931 confirmed cases in 2018, India had 248 cases in 2004 and Canada had 100 cases in 1995 (Arquilella *et al.*, 2019), in China 1.9% through PCR testing (Lass *et al.*, 2022) and in 15% of water sources in Serbia (Cirkovic *et al.*, 2020).

The presence of *T. gondii* oocyst contamination in the environment is inseparable from the presence of cats. The results of field observations during sampling, almost everywhere found stray cats (without owners) in the environment. The nature of cats that defecate anywhere in the environment, on the ground, grass and sand and supported by rain will facilitate the spread of oocysts to the environment. Cats are a source of transmission of toxoplasmosis in the environment because only in cats the process of oocyst formation occurs (Dubey, 2021). The results of the study showed that *T. gondii* contamination was found in all areas of the sampling location. The percentage of *T. gondii* oocyst contamination in Buduran, Sidoarjo, Candi, Wonoayu and Jabon Districts were 21% (3/14), 41% (5/12), 41% (5/12), 30.7% (4/13) and 33.2% (4/12). This difference is possible due to the different soil environments. In general, the soil conditions in Sidoarjo are delta soil consisting of mud, sand, organic matter and minerals. Structure of delta soil contains a lot of water (moist) which is a good place for oocysts to survive in the soil. The presence of minerals and surfactants dissolved in water (water chemistry) also affects the resistance of the oocyst wall (Kinsey *et al.*, 2023). The results of this study can be used as

information that water sources around goat and sheep farms are contaminated with *T. gondii* oocysts which can be transmitted to goats and sheep that are raised. This can occur if drinking water is not given through a certain treatment before being given to livestock. As a prevention, it can provide education on the importance of the presence of unowned cats in the environment as a source of transmission.

CONCLUSION

Drinking water sources on goat and sheep farms in Sidoarjo district have been contaminated with Toxoplasma oocysts.

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AUTHORS' CONTRIBUTIONS

RIMM, M and EPH planned and conducted sample examination and data analysis. LTS, HP and WPL reviewed and provided input on the article. TH, RV, RY and GG assisted in water sampling. HP assisted in sample preparation and examination.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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ETHICAL APPROVAL

The study was formally approved by Animal Care and Use Committee of the Faculty of Medicine, Universitas Airlangga, under reference number 1.KEH.098.07.2024. This approval confirms that the research adhered to established ethical standards regarding animal welfare and data management.

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