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# Comparison of Microscopic and PCR for Detection *Giardia* sp. in the Human Fecal Sample at Bedog Watershed, Sleman, DIY

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## Abstract

Giardia sp. is a gastrointestinal protozoan that is common in mammals and causes giardiasis. Detection of parasitic infections in stool samples can use different methods such as identification of Giardia and trophozoite cysts using a light microscope (saline and iodine) and gene amplification gdh. The aim of this study was to compare the detection of direct microscopic Giardia and PCR in healthy people at risk for Giardiasis in the Bedog watershed, Sleman, DIY. The results of the examination using positive Giardia samples from microscopy were obtained at 4% (4/10). While the PCR results are 7% (7/100). The microscopic method and PCR did not have a significant difference in PCR so that certain microscopic conditions can still be recommended as a basic method in detecting Giardia cysts and trophozoites. The sensitivity and specificity of the direct microscope were 96.9%, and 100%, respectively. The sensitivity and specificity of molecular analysis (PCR) were 97.14% and 100%, respectively. Although PCR detection is more specific than microscopic, in this case, the microscope method can still be used as an initial detection method. While the important advantages of PCR testing, its ability to directly distinguish between different Giardia genotypes, will help deal with cases of Giardiasis. The results of this study indicate that confirmation using the PCR technique can strengthen microscope detection.

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## 1. Introduction

Giardiasis is a disease caused by Giardia sp. Protozoa. Humans and infected animals will secrete cysts with feces and can last for several months. Giardia has a genetic collection of seven collections (A-F), but genotypes A and B can infect humans. In humans, giardia infection through several methods, namely ingestion of cysts through polluted water or direct contact between people. In the digestive system, the cyst is excised and secretes

trophozoites. Furthermore, the trophozoite appears as a protozoan flagella which is shaped like a pear with 2 nuclei [1-5].

Giardiasis is a common disease and can cause outbreaks in various regions [1,6-8]. There are two methods for screening for Giardia infection: direct wet microscopy and molecular methods (Polymerase Chain Reaction / PCR) [1,9-11]. The direct method of wet microscopy takes a long time, is tiring, and requires staff who are experienced in accurately identifying cysts in stool samples [2]. The results of detection using this method for diagnostic techniques have been reported for up to 500,000 cysts per gram of impurity [1, 13]. However, variations of Giardia cause undiagnosed infections, other than that there are different reports on direct methods sensitivity [14, 15]. The diagnosis of Giardiasis in individuals who own livestock and live along the Bedog River Basin plays an important role in controlling Giardiasis. A method commonly used to detect Giardia cysts or trophozoites is microscopic techniques in fresh stool samples and Polymerase Chain Reaction (PCR). One method used to detect Giardia, easy to do, and more sensitive than a microscope is PCR, but this method requires high reagents and costs compared to microscopic directly [9, 16]. Both of these t methods can be used to detect Giardia in humans and animals [9,13,17-19]. The aim of this study was to investigate the prevalence of Giardiasis and compare examination techniques (direct microscopy and PCR) in people living in high-risk areas (Bedog Watershed, Sleman, DIY, Indonesia).

## 2. Materials and Methods

### 2.1. Specimen collection

This study was approved by the review board of the Institutional Ethics committee of the Faculty of Medicine, Universitas Sebelas Maret, Indonesia. Stool samples were collected from individuals who have livestock and live along the Bedog watershed. A total of 100 stool samples were included in this trial. Stool samples are stored at 4 °C with preservatives. This research was conducted after obtaining Ethical approval from the University.

### 2.2. Direct microscopy

Direct microscopy examination was performed on each stool sample. The stool samples collected were then washed using PBS which aims to remove impurities. Examination of stool samples was made by means of fecal smears and dripping using normal saline

and iodine. The presence of trophozoites and Giardia sp cysts was examined under a light microscope with 100X and 400X magnification.

### 2.3. DNA extraction

DNA was extracted from positive stool samples Giardia sp preserved at -20 ˚C using the QIAamp DNA Mini Stool Kit (Qiagen, Hilden, Germany), according to manufacturer's instructions.

### 2.4. PCR assays

Molecular diagnosis of Giardia was performed using the gdh gene (glutamate dehydrogenase) with the primary pair GL-MT-F (Forward) CTCCGCTTCCACCCCTCT; GL-MT-R (Reverse) TGCCTCTGGAGCTCGGTC [20]. The eluted DNA is then amplified in the 188-bp region of the GHD gene according to Shin et al., 2016 [20]. In each reaction, negative (mix + water) controls were added. PCR was performed using Thermocycler (Biorad) and PCR conditions as follows. DNA templates (1-3 µl), 15 µl of 2 × premix PCR (2 × My Taq HS Red Mix, Bioline, Bioexpress), and 5 µl of the primary mixture (consisting of 10 pmol primers in each parasite) mixed with distilled water until a total volume of 25 µl. The PCR reaction for the negative control was reacted only with the primer, without protozoa DNA. At this time, the specificity and sensitivity of each primer were previously tested and confirmed using single PCR amplification. The PCR amplification protocol consisted of 5 minutes at 95˚C for pre-denaturation, 20 cycles of denaturation at 95˚C for 30 seconds, annealing at 65˚C for 40 seconds, and extension at 72˚C for 1 minute. This was followed by 25 cycles of subsequent denaturation at 95˚C for 30 seconds, annealing at 61.2˚C for 40 seconds, and extension at 72˚C for 1 minute. The reaction was omplete with a final extension of 5 minutes at 72˚C. The PCR product was confirmed by CSL Runsafe, Cleaver Scientific) and photographed with UV transillumination, the gel documentation system after loading 1% gel Agarose, LE, Promega) -TBE.

## 3. Results and discussion

A total of 100 stool samples were examined using direct microscopy and PCR methods (Table 1). PCR analysis identified a total of 7 positives (7% of the test sample). Microscopy detected a total of 4 positive (4%) of 7 identified by PCR.

Table 1: Comparison of PCR versus microscopic detection of *Giardia*.

| Direct Microscopy | PCR | | | | PCR | | Direct Microscopy | |
|---|---|---|---|---|---|---|---|---|
| | | Positive | Negative | Total | Sensitivity | Specificity | Sensitivity | Specificity |
| | Positive | 4 | 0 | 4 | | | | |
| | Negative | 3 | 93 | 96 | 97.14% | 100% | 100% | 96.9% |
| | Total | 7 | 93 | 100 | | | | |

The results of the examination using the microscopy method directly showed a sensitivity of 100% and specificity of 96.9% compared to using the PCR method with a sensitivity of 97.14% and specificity of 100% (Table 1). In the direct microscopy method, it took about 10 for making slides. However, the interpretation of results required considerable expertise from staff and had considerable experience. In contrast to the PCR method, preparation time was required long enough starting from total DNA extraction, PCR amplification, and gel analysis, this required a total time of 4.5 hours for a single sample.

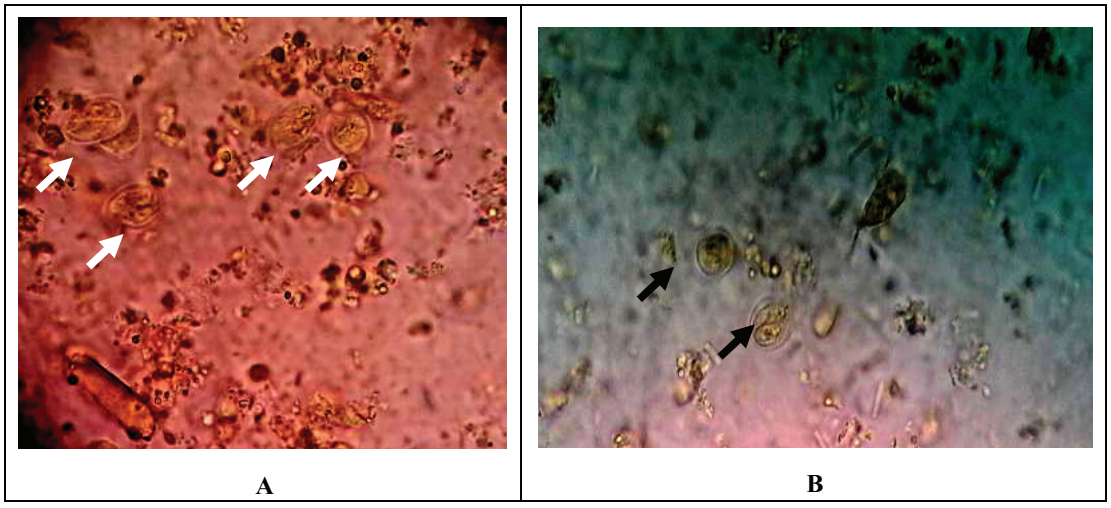


**Figure** 1: Morphology Giardia oocyst using Direct Microscopy by light microscopy at 400x magnification (A and B).

We compared direct microscopy (iodine and saline) and PCR tests to detect Giardia in individuals who owned livestock and lived along the Bedog watershed. From the results of the research on the two methods above, the PCR amplification method is a clear choice to improve Giardia detection from feces, the area is an area that is at risk of Giardiasis. The morphological descriptions of Giardia at the cyst stage in human feces are shown in Figure 1 A and B. This stage can be found on direct microscopic examination (iodine and NaCl) with 400x magnification. In addition to the direct microscopic examination, confirmation is needed to confirm the presence of the GHD gene. The results of both were not different; this can be proven by comparing the results of the direct

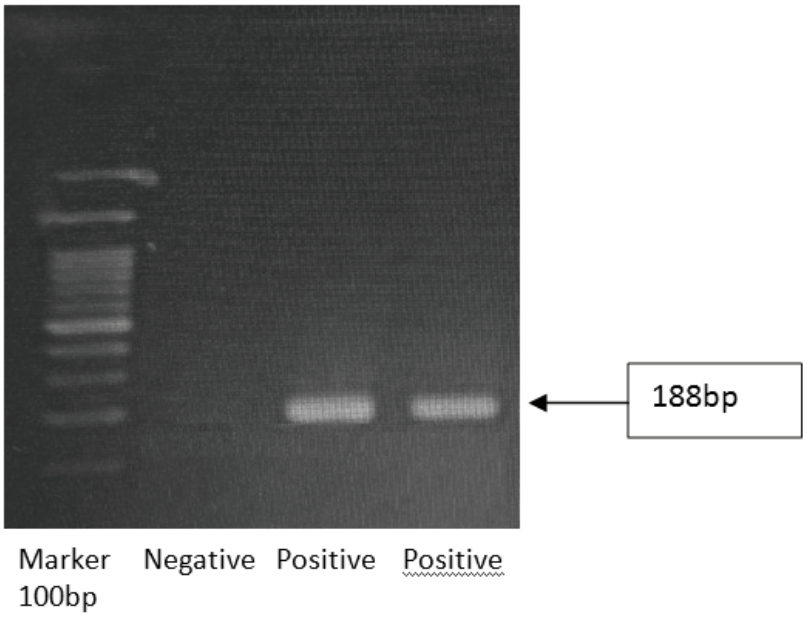


**Figure** 2: Gel Electroforesis Negative dan Positive sample.

microscopic examination and the same PCR results. However, there were 3 positive results from microscopic examination but after negative PCR was confirmed, this was a false positive. This has several reasons because the number of cysts is not much, which amplification effects.

The Polymerase Chain Reaction (PCR) method is currently widely used for the diagnosis of giardiasis, because it has a high sensitivity and specificity compared to direct microscopy. In addition, the PCR method can also detect Giardiasis infection in patients with low parasitic cysts. Using microscopic diagnostic techniques directly and PCR simultaneously will get real positive results [15, 17].

## 4. Conclusions

The results of this study indicate that confirmation using the PCR technique can directly strengthen microscope detection. Although microscopic examination requires the expertise of experienced staff, it is economically cheaper and faster to diagnose Giardia and can detect other parasites; therefore, it must be used as the first choice.

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## References

[1] Dunn N and Juergens, A L 2018 *Giardiasis* in *StatPearls* Treasure Island.

[2] Soares R and Tasca T 2016 *J. Microbiol. Methods* **129** 98-102

[3] Einarsson E, Ma'ayeh S and Svard S G 2016 *Curr. Opin. Microbiol* **34** 47-52

[4] Cama V A and Mathison B A 2015 *Clin. Lab. Med* **35** 423-44

[5] Huang D B and White A C 2006 *Gastroenterol Clin. North. Am* **35** 291-314

[6] Rodriguez-Morales A J, Granados-Alvarez S, Escudero-Quintero H, Vera-Polania F., Mondragon-Cardona A, Diaz-Quijano F A *et al* 2016 *Int. J. Infect. Dis* **49** 204-9.

[7] Manser M, Granlund M, Edwards H, Saez A, Petersen E, Evengard B *et al* 2014 *Clin. Microbiol. Infect* **20** 65-71

[8] Adam EA, Yoder JS, Gould LH, Hlavsa MC, and Gargano J W 2016 *Epidemio.l Infect* **144** 2790-801

[9] El-Nahas H A, Salem D A, El-Henawy A A, El-Nimr H I, Abdel-Ghaffar H A and El-Meadawy A M 2013 *Cytometry. B. Clin. Cytom* **84** 44-9

[10] Hawash Y, Ghonaim M M, and Al-Shehri S S 2016 *Korean J. Parasitol.* **54** 1-8

[11] Hijjawi N, Yang R, Hatmal M, Yassin Y, Mharib T, Mukbel R*, et al* 2018 *Exp. Parasitol* **185** 23-28

[12] Adeyemo F E,Singh G,Reddy P, and Stenstrom T A 2018 *Acta Trop* **184** 15-28

[13] Johnston S P, Ballard M M, Beach M J, Causer L and Wilkins P P 2003 *J Clin Microbiol* **41** 623-6

[14] Gotfred-Rasmussen H, Lund M, Enemark H L, Erlandsen M and Petersen E 2016 *Diagn. Microbiol. Infect. Dis* **84** 187-90

[15] Asher A J, Waldron L S and Power M L 2012 *Parasitol. Res* **110** 853-8

[16] Ignatius R, Gahutu J B, Klotz C, Steininger C, Shyirambere C, Lyng M *et al* 2012 *PLoS. Negl. Trop. Dis* **6** 1677

[17] Koehler A V, Jex A R, Haydon S R, Stevens M A and Gasser R B 2014 *Biotechnol Adv* **32** 280-9

[18] Johnston A R, Gillespie T R, Rwego I B, Mclachlan T L, Kent A D and Goldberg T L 2010 *PLoS Negl. Trop. Dis* **4** 683

[19] Helmy Y A, Klotz C, Wilking H, Krucken J, Nockler K, Von Samson-Himmelstjerna G *et al* 2014 *Parasit Vectors* **7** 321

[20] Shin J H, Lee S E, Kim T S, Ma D W, Chai J Y and Shin E H 2016 *Korean J Parasitol* **54** 631-36.