Rapid clearance of *Giardia lamblia* DNA from the gut after successful treatment

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Abstract

To assess the time it takes for a real-time PCR to become negative after treatment of a *Giardia lamblia* infection, we evaluated two consecutive follow-up samples from 75 infected patients. Approximately 1 week after treatment all samples tested negative, indicating rapid clearance of parasitic DNA after successful treatment.

Keywords: Clearance, follow-up, Giardia, real-time PCR, treatment

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Introduction

*Giardia lamblia* is a protozoan parasite causing diarrhoea in individuals worldwide. The estimated prevalence in industrialized countries is c. 2% for adults and 6–8% for children [1]. Three Dutch studies detected *G. lamblia* DNA in 5.8–11.1% of samples taken from patients presenting with gastrointestinal symptoms [2]. Traditionally, microscopy is the diagnostic method of choice, although sensitivity is known to vary depending on the number of samples examined, the experience of the microscopist and the use of concentration techniques [3]. Direct fluorescent antibody (DFA) and ELISA-based methods are sensitive and cost-effective [4,5], although, like microscopy, time consuming and laborious. Lately, real-time PCR was introduced to the clinical microbiology laboratory for diagnosis of parasitic intestinal infections. It has a significantly higher detection rate for *G. lamblia* compared with traditional microscopy and allows automation and simultaneous detection of different targets [2,3,6]. A highly relevant question accompanying molecular diagnostics is how long parasitic DNA can be detected after successful treatment. In patients with persistent symptoms the differentiation between therapy failure and detection of DNA of non-viable parasites is important for further treatment. *G. lamblia* is non-invasive and attaches to the mucosa of the small intestine where it undergoes asexual replication. As the estimated turnover time of the intestinal mucosa is 2–4 days, we hypothesize that the real-time PCR on faecal samples becomes negative within 1 week after successful treatment.

To confirm this hypothesis we performed a prospective study at the St Elisabeth hospital Tilburg, the Netherlands. Between January and October 2013, inpatients and outpatients with a PCR-based diagnosis of *G. lamblia* were considered eligible to participate if they had not received treatment so far. Patients were informed of the diagnosis by their general practitioner or attending specialist, who received information concerning the study and advice on therapy (metronidazole; 2 g once daily for 3 days for adults, 50 mg/kg once daily for 3 days for children) from the consultant microbiologist. Patients were asked to return consecutive stool samples after treatment. Follow-up sample 1 (FU1) was requested at the end of antibiotic treatment (EAT), follow-up sample 2 (FU2) 7 days after FU1, and an optional follow-up sample 3 (FU3). 1 month after FU1, was requested only if *Giardia* DNA was detected in follow-up sample 2. Automated nucleic acid extraction using QIAsymphony [7] and a multiplex real-time PCR for the detection of *G. lamblia*, *Entamoeba histolytica* and *Cryptosporidium* spp. [8,9] was performed on all samples within 24 h after receipt of the sample. Statistical analysis was performed by using SPSS version 19.0. (IBM Corp., Armonk, NY, USA). Comparison of continuous variables was performed by using the Mann–Whitney U-test, and comparison of binary data by using Fisher’s exact test. This study was approved by the ethics committee of the St Elizabeth Hospital, Tilburg, the Netherlands.

During the study period the multiplex PCR was performed on samples from 2307 persons. One hundred and eleven patients were found to be eligible for inclusion based on *Giardia* DNA detection (positivity rate, 4.8%). Seventy-five (67.6%) patients were willing to participate. There was no difference in the age (p 0.818) and initial Cycle threshold (Ct) value (p 0.415) of the participating patients compared with the
36 non-participating patients. For 10 patients no FU2 samples were received so only FU1 data were included in the analysis. Giardia DNA was detected in 30 of 75 FU1 samples (40.0%) and in none of the 65 FU2 samples (0.0%) (Fig. 1), therefore no FU3 samples were requested. The median time between EAT to FU1 was 1 day (range 0–22 days) and the median time between FU1 and FU2 was 7 days (range 4–22 days). Ninety per cent of all samples were received within 4 and 10 days, respectively. The absence of Giardia DNA in all FU2 samples indicates a rapid clearance after treatment.

The Ct-values of the initial samples from patients who tested negative at FU1 were significantly higher than those for patients who tested positive at FU1 (median 27.3 vs 23.0, \( p < 0.001 \)), indicating a lower parasitic load. Also the time interval between EAT and FU1 differed significantly between the two groups (median 2 days vs 1 day, \( p = 0.04 \)). Age did not influence the probability of a positive FU1 test (OR < 18 years vs adults = 1.27, \( p = 0.48 \)).

Using a multiplex PCR, Cryptosporidium spp. DNA was detected in 1.0%, 1.7% and 1.8% of the initial samples, FU1s and FU2s, respectively. E. histolytica DNA was not detected during the study period.

The rapid clearance of Giardia DNA in all patients is in contrast to findings reported by Mejia et al. [6]. They described that c. 65% of their treated study population (albendazole/ivermectin for 3 days) cleared Giardia DNA after 21 days. This difference might be explained by a non-optimal treatment regime or by the greater risk of re-infection due to a high prevalence in the study area (Ecuador).

In other studies, 20% treatment failure after metronidazole therapy, possibly caused by drug resistance, was reported, especially in patients who acquired their infection in Asia [10–12]. Although in the daily routine we sometimes encounter Giardia infections in patients who do not respond to metronidazole treatment, the 100% clearance rate found in the present study period suggests that no treatment failure occurred. This short-course molecular follow-up study for G. lamblia infection can not exclude the possibility of intermittent Giardia shedding; however, all patients who had negative FU1 samples remained negative at FU2.

A limitation of our study is the treatment delay. Even though we contacted the attending physician immediately after PCR results were authorized, the median time between the initial diagnosis and EAT was 5.5 days (range 2–19 days). As a result of this treatment delay, some patients may have already cleared G. lamblia as a natural course of infection, resulting in negative FU1 samples. However, the time between the initial diagnosis and the end of treatment was comparable in patients

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**FIG. 1.** Flow-chart of inclusion, number of follow-up samples received and test results of this study.
who tested positive and patients who tested negative at FU1 (median 5 vs 6 days, p 0.249).

Our study shows that shortly after treatment Giardia DNA is undetectable in the stool, making a positive multiplex PCR test 1 week after treatment a strong indication of renewed or on-going infection. Probably, this does not only apply to G. lamblia but also to other non-invasive parasitic infections.

Transparency Declaration

The authors declare no conflicts of interest.

References