Detection of intestinal protozoa in paediatric patients with gastrointestinal symptoms by multiplex real-time PCR

L. Maas[1], J. W. Dorigo-Zetsma[2], C. J. de Groot[1], S. Bouter[2], F. B. Plötz[1] and B. E. van Ewijk[1]

1) *Department of Paediatrics, Tergooi Hospitals and* b) *Department of Medical Microbiology, Tergooi Hospitals, Hilversum, The Netherlands*

Abstract

The performance of a multiplex real-time PCR for the detection of *Blastocystis*, *Dientamoeba fragilis*, *Giardia lamblia*, *Cryptosporidium* species and *Entamoeba* species in faecal samples was evaluated in an observational prospective study. Paediatric patients (0–18 years) presenting with gastrointestinal symptoms and suspected of having enteroparasitic disease were included. A questionnaire on gastrointestinal symptoms and the chosen treatment was completed at the start of the study and after 6 weeks. Of 163 paediatric patients (mean age, 7.8 years), 114 (70%) had a PCR-positive faecal sample. *D. fragilis* was detected most frequently, in 101 patients, followed by *Blastocystis* in 49. In faecal samples of 47 patients, more than one protozoan was detected, mainly the combination of *D. fragilis* and *Blastocystis*. Reported gastrointestinal symptoms were abdominal pain (78%), nausea (30%), and altered bowel habits (28%). Eighty-nine of the PCR-positive patients were treated with antibiotics. A significant reduction in abdominal pain was observed both in treated and in untreated patients. This study demonstrated that multiplex real-time PCR detects a high percentage of intestinal protozoa in paediatric patients with gastrointestinal symptoms. However, interpretation and determination of the clinical relevance of a positive PCR result in this population are still difficult.

Keywords: *Blastocystis*, *Dientamoeba fragilis*, gastrointestinal symptoms, intestinal protozoa, multiplex real-time PCR, paediatrics, RT-PCR
Original Submission: 17 May 2013; **Revised Submission:** 29 August 2013; **Accepted:** 30 August 2013
Editor: E. Bottieau
Article published online: 17 October 2013
Clin Microbiol Infect 2014; **20:** 545–550
doi: 10.1111/1469-0691.12386

Corresponding author: B. E. van Ewijk, Department of Paediatrics, Tergooi Hospitals, Box 10016, 1201 DA Hilversum, The Netherlands
E-mail: bvanewijk@tergooiziekenhuizen.nl

Introduction

Gastrointestinal symptoms, such as abdominal pain, nausea, acute or chronic diarrhoea, and altered bowel habits, are frequently seen in paediatric patients. Among several other causes, intestinal protozoa may be involved. However, the actual role of protozoal infections in cases with gastrointestinal symptoms, and therefore the relevance of detection of intestinal protozoa, is a subject of discussion [1–3].

In The Netherlands, the routine diagnostic procedure for detection of intestinal protozoa consists of microscopy on two sodium acetate formalin-preserved stool specimens and on one unpreserved specimen in a so-called triple faeces test (TFT) [4]. Although the TFT has shown to be an effective tool for the detection of intestinal parasites [4], it requires considerable effort. The patient has to collect three stool samples on three consecutive days, and the microbiological laboratory has to examine three samples microscopically. The complexity of the TFT procedure might be one of the reasons why only a limited amount of data on the prevalence, clinical characteristics and treatment outcome of parasitic gastrointestinal illness in paediatric patients is available. Real-time PCR has recently been shown to be a sensitive and specific diagnostic alternative for the detection of intestinal protozoa, and some authors recommend its routine use [5–7]. It is less labour-intensive, and has comparable or higher sensitivity with only one stool sample instead of three, making it an attractive alternative to microscopy. However, no clinical data on the implementation of real-time PCR in daily paediatric practice are available in these or other studies.

This prospective, observational and daily practice study was undertaken to identify intestinal protozoa in faeces of paediatric patients with gastrointestinal symptoms by use of a multiplex real-time PCR and to follow up clinical features 6 weeks after inclusion.

Patients and Methods

The study was carried out in the outpatient paediatric department of a general teaching hospital and in the practices of ten collaborating general practitioners (GPs). Patients were included during a 6-month period, from September 2010 to March 2011. The ethical committee of the hospital approved the study.

Study design

Paediatric patients (0–18 years) with any presentation of gastrointestinal symptoms lasting for >2 weeks and/or paediatric patients clinically suspected of having a parasitic gastrointestinal illness by the treating paediatrician or GP were included if their physician decided to perform PCR to detect intestinal parasites in faeces. Paediatric patients diagnosed with other common causes of gastrointestinal symptoms were excluded. This included the suspicion and detection of gastrointestinal bacteria and viruses, chronic gastrointestinal morbidity (such as inflammatory bowel disease or coeliac disease), recent use of antibiotics (in the past 6 weeks), and immunocompromised status.

All paediatric patients and/or their parents completed a questionnaire about the characteristics of the gastrointestinal symptoms. The questionnaire consisted of questions on the presence of abdominal pain, nausea, acute diarrhoea (more than three loose stools a day, present for <14 days), chronic diarrhoea (diarrhoea lasting for >14 days), altered bowel habits (defined as a change in stool pattern other than diarrhoea), weight loss, vomiting, and anal itching. The severity of abdominal pain was scored on a validated paediatric visual analogue scale (VAS), which scores the severity of pain on a scale from 0 to 10 [8].

After completion of the questionnaire, a multiplex real-time PCR was performed for *Blastocystis*, *Dientamoeba fragilis*, *Giardia lamblia*, *Cryptosporidium* species and *Entamoeba* species on a stool sample collected at home (T0). A week after the first visit (T1), the treating physician communicated (by telephone or at a doctor's visit) the PCR results. As there is a lack of evidence concerning both the criteria for starting treatment and the ideal drug regimen, the choice of whether or not to treat (and with which type of antibiotic) in the case of a positive PCR result was left to the treating physician. Details on treatment were registered. Six weeks after the first visit (T6), all treated and untreated paediatric patients and/or their parents filled out the same questionnaire as on T0 in order to enable follow-up of clinical characteristics, the effect of treatment, or the natural course of the symptoms.

Multiplex real-time PCR for intestinal protozoa

For the multiplex real-time PCR *c.* 200 mg of unpreserved faeces was dissolved in 400 μL of lysis buffer (DXL; Qiagen, Hilden, Germany), and, after storage at −20°C overnight, was used for DNA extraction. Prior to automated DNA extraction, phocin herpesvirus (PhHV-1) was added to the faecal sample to serve as an internal control for determining the efficiency of the PCR and detecting inhibition in the sample [9]. Detection of the five protozoa was performed in two separate PCR reactions per DNA sample. In one reaction, a PCR for *G. lamblia* and *D. fragilis*, including PhHV-1, was performed as described previously [10,11]. In a separate assay, *Blastocystis*, *Cryptosporidium* species, and *Entamoeba* species, including PhHV-1, were amplified [12]. The analytical sensitivity and specificity of the PCRs used have been validated at the Leiden University Medical Centre and Tergooi Hospitals (The Netherlands) [10,11], and confirmed after standardized adjustments to the analysis parameters in RotorGene software (Qiagen). Negative extraction and positive DNA controls for each pathogen were included in all PCR runs.

Statistical analysis

Dichotomous and categorical variables were compared by use of the χ^2-test, and continuous data were analysed with non-parametric tests as applicable. All statistical analyses were performed with SPSS version 20 (SPSS, Chicago, IL, USA). A p-value of <0.05 was accepted as statistically significant. Data are expressed as median and range unless stated otherwise.

Results

A total of 171 paediatric patients (61% with gastrointestinal symptoms lasting for >2 weeks and 39% with clinical suspicion of parasitic gastrointestinal illness) participated in the study; eight patients were excluded because they met one of the exclusion criteria. Real-time PCR was positive in 114 of 163 (70%) of the paediatric patients (Table 1). Because of loss to

TABLE 1. PCR results

PCR-positive	PCR-negative
n = 114 (70%) Ten lost to follow-up	*n* = 49 (30%) Five lost to follow-up