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qRT-PCR is a Highly Sensitive, Highly Specific Diagnostic Adjunct to H&E Microscopy to Detect Giardia Using Formalin-Fixed Paraffin-Embedded Tissue

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Abstract

Background: The diagnosis of Giardia spp. may pose a challenge on routine Hematoxylin and Eosin (H&E) sections of small intestinal biopsy specimens. Additionally, clinicians may not order additional laboratory testing for Giardia at the time of the biopsy due to the frequently nonspecific clinical presentation. We selected cases diagnosed as positive for Giardia by H&E microscopy and tested the corresponding Formalin-Fixed, Paraffin-Embedded (FFPE) tissue using a quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) to evaluate the performance characteristics in detecting Giardia.

Design: We obtained 65 small intestinal biopsy specimens that were diagnosed as positive for Giardia on H&E sections from the years 1991–2012. We then tested the cases for evidence of Giardia by qRT-PCR using the FFPE tissue. Seventy negative control biopsies from the small intestine and colon were also tested. A single case diagnosed as equivocal for Giardia by H&E microscopy was also selected. A review of the medical records for the positive cases was done to determine whether any additional laboratory testing for Giardia spp. had been performed at the time of the biopsy.

Results: Of the 65 biopsy specimens positive for Giardia by H&E, 64 also tested positive by qRT-PCR, resulting in a sensitivity of 98.5%. All 70 negative control cases were also negative by qRT-PCR, resulting in a specificity of 100%. The equivocal case tested negative for Giardia by qRT-PCR. Pertinent medical records were available for 21 of the Giardia-positive cases. Of these, only one case had a corresponding stool ova and parasite examination, which was also positive for Giardia. The remaining 20 cases did not have any additional laboratory testing performed for Giardia at the time of the biopsy.

Conclusion: qRT-PCR using FFPE tissue is a useful, highly sensitive and highly specific molecular adjunct to H&E histology in the diagnosis of Giardia, especially in equivocal cases. qRT-PCR may help facilitate an accurate diagnosis in cases with nonspecific clinical presentations for which an endoscopic examination with biopsy is performed.

Keywords: *Giardia;* qRT-PCR; Formalin-Fixed Paraffin-Embedded Tissue; Small Bowel; Diagnostics

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Highlights

- Diagnosing *Giardia* can be challenging on routine H&Es of small intestinal biopsies
- qRT-PCR using FFPE tissue is a highly sensitive and specific for *Giardia*
- qRT-PCR using FFPE tissue is a useful molecular adjunct in the diagnosis of *Giardia*
- qRT-PCR assists in diagnosing nonspecific gastrointestinal cases

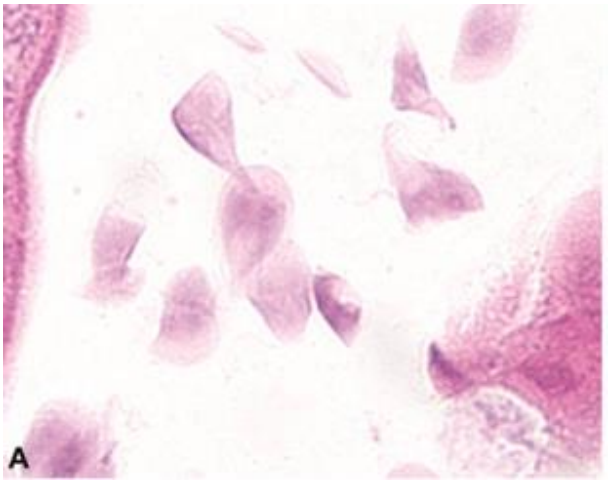
Introduction

Giardia spp. are single-celled protozoan organisms that parasitize the upper small intestine in humans. In some cases, patients may manifest little to no clinical symptoms. In other instances, severe diarrhea and malabsorption may ensue, prompting medical attention. Risk factors for infection include travel to endemic areas, daycare, exposure to contaminated water, outdoor recreational activity, and men who have sex with men [17]. The differential diagnoses in these cases may include a myriad of noninfectious etiologies including adverse reactions to medications, food intolerance, microscopic colitis, and inflammatory bowel disease. Infectious causes may include numerous viral or bacterial organisms as well as parasites.

Giardia is transmitted via fecal-oral contact or through the consumption of contaminated water or food, and possesses two distinct stages of development: the encapsulated cyst form and the trophozoite form [3]. The cysts are found in contaminated water and food and are ingested by the host. Exposure to the acidic gastric environment prompts excystation in the duodenum where the cysts develop into motile, flagellated trophozoites. The trophozoites are the vegetative forms of the organism responsible for disease manifestation [3, 12, 13].

The trophozoite forms are identifiable in routine hematoxylin and eosin (H&E)-stained paraffin sections of small intestine biopsy specimens. The trophozoites have a characteristic pear-shaped, binucleate appearance on H&E sections (Figure 1A). In cross-section, the organisms may appear lenticulated and can resemble epithelial debris (Figure 1B and 1C). Although villous atrophy and crypt hyperplasia may be seen histologically, infection does not always elicit a robust immune response, and frequently the small intestinal villous architecture is well preserved [13]. For these reasons, giardiasis may commonly be missed on routine microscopic examination.

Figure 1A & B: Trophozoites of *Giardia* in the intervillous spaces, H&E section (60x magnification)



B: 20x magnification

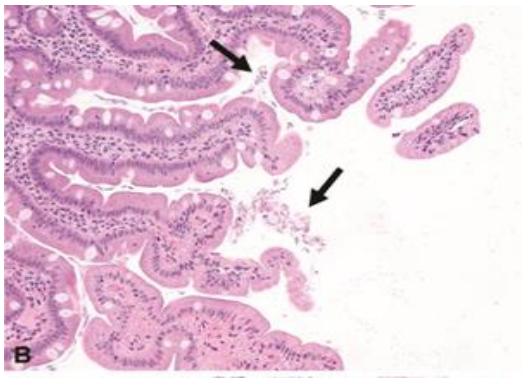
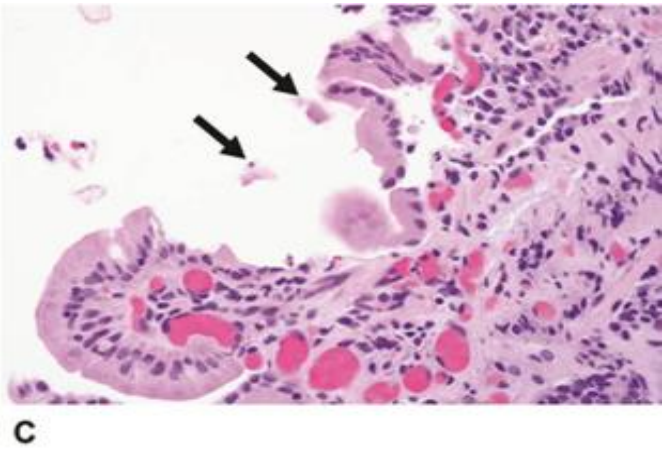


Figure C: Epithelial debris in the intervillous spaces, H&E section



In this study, we selected cases of *Giardia* diagnosed on H&E-stained sections of small bowel biopsies. We then utilized quantitative real-time polymerase chain reaction (qRT-PCR) on cut paraffin sections of the biopsy blocks to better characterize the role of molecular methods as an adjunct to H&E in the detection of *Giardia*.

Materials and Methods

With the approval of the Institutional Review Board, a search of our electronic laboratory databases was performed to identify 65 small bowel biopsy specimens in which *Giardia* had been detected by histopathology on routine H&E sections. The accession years of these cases ranged from 1991–2012. Seventy additional cases, including normal colon and small intestine (n=25), active colitis (n=25), and active duodenitis (n=20), were identified for use as negative controls. The accession years of these cases ranged from 2009–2012. One case reported as indeterminate was identified, and was included as an equivocal case. Two pathologists reviewed all of the cases (EC and JL).

In total, 136 paraffin blocks were used in testing for *Giardia* by qRT-PCR. From each block, 5 x 10 μM thick scrolls of tissue were cut and DNA extractions were performed according to the manufacturer's specifications (QIAamp DNA FFPE Tissue Kit, Qiagen, Valencia, CA, USA). An internal reaction control was then added to the extracted DNA and the specimens were analyzed by qRT-PCR using reagents QIAGEN HotStarTaq Master mix (250 units), catalog no. 20344 and primers/probes from Integrated DNA Technologies (Coralville, Iowa; *forward primer* GAC GGC TCA GGA CAA CGG TT, *reverse primer*

TTG CCA GCG GTG TCC G, *probe* 56-FAM/CC CGC GGC G/Zen/G TCC CTG CTA G/3IABkFQ) on the LightCycler 2.0 (Roche, Indianapolis, IN). The cutoff point was set at 40ct. Results were reported as either positive or negative for *Giardia* at these specific cutoff values.

A review of the available medical records was performed to determine whether additional concomitant microbiological studies for *Giardia* were ordered at the time of biopsy.

Results

qRT-PCR results on histologically positive cases

As shown in Table 1, 65 small intestine biopsies positive for *Giardia* by histopathology, 64 were also positive by qRT-PCR. One case diagnosed as positive for *Giardia* by histopathology had a negative result by qRT-PCR. These results reflected a sensitivity of 98.5%.

Medical records were available for 21/65 of the *Giardia*-positive cases. Only one out of the 21 cases had a corresponding positive stool Ova and Parasite (O&P) examination. This case was also positive for *Giardia* by qRT-PCR. The remaining 20 cases did not have any additional laboratory testing performed for *Giardia* at the time of the biopsy.

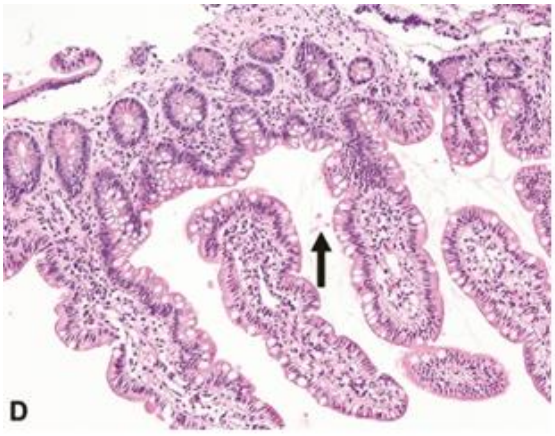
qRT-PCR results on histologically negative cases

All 70 cases that were negative for *Giardia* by histopathology also had negative results by qRT-PCR. The specificity for qRT-PCR was 100%.

qRT-PCR results on histologically equivocal case

As shown in Table 1, one small intestine biopsy was diagnosed as suspicious for *Giardia* by histopathologic examination. Reexamination of this case revealed a few foci of eosinophilic to amphophilic material within the intervillous spaces (Figure 1D). qRT-PCR performed on this specimen produced a negative result.

Figure D: Case equivocal for *Giardia*, H&E section.



Discussion

Several diagnostic methods are available for the detection of *Giardia*. These include enzyme-linked immunosorbent assays (EIA), direct fluorescent-antibody tests (DFA), immunochromatographic lateral flow immunoassays, and stool O&P examination. Additionally, molecular detection methods such as PCR have also been investigated [2, 6, 9, 15, 16].

Traditionally, manual stool O&P exams have been used for the diagnosis of *Giardia*. However, this method is time consuming, labor intensive, and dependent on the skill of the microscopist. Additionally, *Giardia* cysts are known to be shed sporadically in infected individuals. In fact, stool O&P examination of a single stool specimen has a sensitivity of only 50–70%, and it is recommended that specimens be collected during a ten-day timeframe [7, 14, 15].

In many institutions, rapid immunoassays have largely replaced traditional stool O&P examination as a screening modality for *Giardia* [1, 4, 5, 8, 10, 11, 15]. Immunoassays have been reported to have a similar sensitivity and specificity compared to O&P examination. Additionally, immunoassays have the advantage of speed and cost-efficiency compared to traditional microscopy [14, 15].

Infection with Giardia often presents nonspecifically, and stool O&P exams and other specific microbial detection tests may not be ordered at the time of endoscopy. In these situations, *Giardia* may be diagnosed by morphology on microscopic H&E-stained slides alone. In fact, in this study, only one out of 21 cases (5%) for which laboratory information was available had a corresponding microbiological detection test. Epithelial debris, as well as foreign material, may sometimes mimic *Giardia* organisms in H&E-stained sections, potentially posing a diagnostic challenge. In this scenario, using PCR as an ancillary diagnostic tool may be useful.

PCR has previously been performed to diagnose other pathogenic organisms such as *Histoplasma capsulatum*, *Candida albicans*, and several viruses using formalin-fixed paraffin embedded tissue [6, 9, 16, 2]. In one previous study, PCR was shown to have sensitivity to detect *Giardia* comparable to that of immunoassays and O&P examination (98–100%), but has a

lightly decreased specificity (92%) compared to the latter modalities (>97%) [15]. In this study, we demonstrated that qRT-PCR is a rapid, highly sensitive (98.5%) and highly specific (100%) diagnostic adjunct to the detection of *Giardia* by H&E microscopy. A potential explanation for why one case diagnosed as positive by H&E was negative by qRT-PCR may be attributed to an insufficient amount of tissue sectioned for nucleic acid extraction. Additionally, the specimen was archived (accession year 1993), which may have degraded the quality of the nucleic acid.

Conclusions

In diagnostically challenging cases, qRT-PCR is a highly sensitive and specific ancillary molecular test in the diagnosis of *Giardia* spp. in paraffin-embedded tissue. qRT-PCR for *Giardia* may facilitate an accurate diagnosis in clinical cases with nonspecific gastrointestinal presentations and no other laboratory test available.

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Table 1: Summary of Positive and Equivocal Cases

Case number	Age	Gender	Race	Giardia semi-quantitation on H&E (score)*	PCR results (at 40ct)
1	45	Male	White	Positive (2)	Positive
2	32	Male	Black	Positive (2)	Positive
3	44	Female	White	Positive (2)	Positive
4	55	Male	White	Positive (2)	Positive
5	36	Female	White	Positive (2)	Positive
6	29	Female	Black	Positive (2)	Positive
7	51	Male	White	Positive (2)	Positive
8	67	Male	White	Positive (2)	Positive
9	9	Male	White	Positive (2)	Positive
10	3	Male	White	Positive (2)	Positive
11	14	Female	White	Positive (2)	Positive
12	14	Male	White	Positive (1)	Positive
13	10	Female	White	Positive (2)	Positive
14	11	Female	White	Positive (1)	Positive
15	13	Male	Black	Positive (2)	Positive
16	6	Male	White	Positive (1)	Positive
17	17	Female	White	Positive (2)	Positive
18	11	Female	White	Positive (1)	Positive
19	12	Female	White	Positive (2)	Positive
20	10	Female	White	Positive (2)	Positive
21	11	Male	White	Positive (2)	Positive
22	11	Female	White	Positive (2)	Positive
23	14	Female	Black	Positive (1)	Positive
24	2	Male	White	Positive (1)	Positive

25	12	Male	White	Positive (2)	Positive
26	1	Female	White	Positive (2)	Positive
27	10	Male	White	Positive (2)	Positive
28	8	Male	White	Positive (2)	Positive
29	10	Male	White	Positive (2)	Positive
30	17	Female	White	Positive (2)	Positive
31	12	Male	White	Positive (2)	Positive
32	2	Male	White	Positive (1)	Positive
33	16	Female	White	Positive (2)	Positive
34	2	Female	White	Positive (2)	Positive
35	5	Male	White	Positive (1)	Positive
36	7	Female	White	Positive (2)	Positive
37	2	Male	White	Positive (2)	Positive
38	1	Male	White	Positive (2)	Positive
39	6	Male	Black	Positive (1)	Positive
40	10	Male	White	Positive (2)	Positive
41	3	Male	White	Positive (1)	Negative
42	11	Male	White	Positive (2)	Positive
43	4	Female	White	Positive (1)	Positive
44	3	Male	White	Positive (1)	Positive
45	25	Male	White	Positive (2)	Positive
46	1	Male	White	Positive (2)	Positive
47	11	Male	White	Positive (2)	Positive
48	11	Female	White	Positive (2)	Positive
49	9	Male	White	Positive (2)	Positive
50	9	Male	White	Positive (2)	Positive
51	3	Female	White	Positive (2)	Positive
52	16	Male	White	Positive (2)	Positive
53	6	Male	White	Positive (2)	Positive
54	14	Female	White	Positive (2)	Positive
55	3	Female	White	Positive (2)	Positive
56	3	Male	White	Positive (2)	Positive

57	4	Male	White	Positive (2)	Positive
58	2	Male	White	Positive (2)	Positive
59	11	Female	Unknown	Positive (1)	Positive
60	13	Female	Black	Positive (2)	Positive
61	11	Male	White	Positive (2)	Positive
62	5	Male	White	Positive (2)	Positive
63	45	Male	White	Positive (2)	Positive
64	55	Male	White	Positive (2)	Positive
65	56	Female	White	Positive (2)	Positive
66	1	Female	White	Equivocal	Negative