

Intraepithelial *Giardia Intestinalis*: A Case Report and Literature Review

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Abstract: The giardiasis is a neglected parasitic disease. The WHO has estimated more than 280 million of human infections each year; however, intraepithelial giardiasis is a rare entity, there are only 5 reports showing invasive giardiasis.

A pediatric female patient with chronic abdominal pain, diarrhea, and pasty stools, without fever, was seen in the Gastroenterology and Nutrition Service. The stool studies were negative for pathogens and lactose hydrogen breath test was positive. The presumptive clinical diagnosis was giardiasis and the patient was empirically treated with nitazoxanide. But, the patient persisted with abdominal pain and pasty stools.

Endoscopy was indicated to search for *Helicobacter* and *Giardia*. Guardian and patient gave written informed consent. Hematological profile was normal. The endoscopy was performed under general anesthesia and the biopsies and duodenal aspirate were obtained. The microscopic analyses of duodenal fluid showed *Giardia* trophozoites. Electron microscopic analysis was negative for *Helicobacter pylori*, but *Giardia* trophozoites with a typical crescent shape within the tissue were found.

The patient was treated with tinidazole, subsequent tests showed that lactose absorption was normal, stool examinations were negative for *Giardia* and abdominal pain had stopped.

This case suggest that intraepithelial giardiasis could be a common entity but unseen because the giardiasis diagnosis is usually made on fecal samples. Future studies are necessary to determine the role of intraepithelial trophozoites in giardiasis pathogenic mechanisms

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INTRODUCTION

Giardia intestinalis (also known as *G lamblia* or *G duodenalis*) is a successful intestinal parasite that causes waterborne diarrhea in humans and other mammals throughout the world.¹ The WHO has estimated more than 280 million of human infections each year only in Africa, Asia, and America.² In Mexico the seroprevalence is 55.3%.³ Giardiasis can be asymptomatic or symptomatic characterized by foul smelling diarrhea, with abdominal pain that is exacerbated by eating,

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flatulence, anorexia, and weight loss. In patients with chronic giardiasis there are periods with constipation or diarrhea with steatorrhea; nutrients malabsorption, and lactase deficiency.^{4,5} The parasite impairs the children's physical and mental development, especially those in developing countries.⁶ The giardiasis was included in the "neglected diseases initiative" in 2004.⁷

The *G intestinalis* morphological simplicity hides a big genetic variability; using molecular tools, have been described 8 assemblages/genotypes, A to H. Assemblages A (subgenotypes A-I and A-II) and B infect humans and other mammals, C and D mainly live in dogs, E thrive in artiodactyls, F in felines, G was described in rats,^{8,9} and H harbored in marine mammal.¹⁰

The pathogenic mechanism involves trophozoite adhesion to epithelial cells by the ventral suckling disc, using non-specific mechanical forces and/or specific interactions with lectins¹¹ and annexins.¹² The interaction between *Giardia* suckling disc and enterocytes generates a lesion on microvillus border of epithelium,¹³ that alter the ionic cell equilibrium, augmenting the peristalsis and producing diarrhea. There is also an increase in intestinal permeability caused by modifications in the zonula-occludens (ZO) tight-junction.^{14,15} *Giardia* trophozoites have evolved to colonize an adverse environment, therefore, the parasite is able to modify and manipulate its milieu in several ways such as: competition for arginine to reduce the Nitric Oxide (NO) production in epithelial cells;¹⁶ the contact with the *Giardia* variable surface proteins (VSPs) to modulate the protein-protein interactions,¹⁷ as well as zinc/iron depletion¹⁸ and antigenic variation to avoid immune attack.^{19,20} The proteinase secretions protect trophozoites by degrading secreted IgA, or perhaps acting like caspases aimed to promote apoptosis.²¹ Interaction *Giardia*-enterocyte induces goblet cells hyperplasia and consequently produces cellular gaps on the epithelial barrier.²² It has been demonstrated that tight interaction between *Giardia* and Caco2 cells start the modulation in gene expression of the immune response²³ and a complex genomic interactions with down/up gene regulations in *Giardia*.²⁴ All these phenomena, directly or indirectly, could deteriorate the epithelial barrier. In Mexico, there is a predominance of *Giardia* assemblage A in symptomatic or asymptomatic patients,^{25,26} until now, it has been impossible to make an unequivocal differentiation between asymptomatic *Giardia* isolates from those capable to produce clinical symptomatology.²⁷⁻²⁹ Therefore is necessary to think in alternative hypothesis, then we wish to present the case of *Giardia* trophozoites within the intestinal epithelium from a patient with chronic abdominal pain and lactose intolerance, and a review of the literature.

PATIENT AND METHODS

Case Report

A patient of 9.5 year old, with chronic pain abdominal was admitted in the Gastroenterology and Nutrition Service and integrated to: The chronic abdominal pain protocol, authorized

by the Ethical and Research Committee of the Instituto Nacional de Pediatría. The patient had history of diarrhea or pasty stools, neither fever nor vomiting. The feces from patient were processed seeking for pathogenic bacteria and the results were negatives. The microscopic examination looking for cysts and ova after the centrifugation-concentration method³⁰ was negative for cysts and eggs. The lactose hydrogen breath test was positive. The presumptive clinical diagnosis was giardiasis and the patient was empirically treated with nitazoxanide (7.5 mg/kg/12 h/3 days). However, the patient persisted with abdominal pain and pasty stools; therefore, endoscopy was indicated to search for *Helicobacter* and *Giardia*. Hematological profile was: white blood cells 4700/mL, neutrophils 44%, hemoglobin 14.7 g/dL, hematocrit 43.2%, mean corpuscular volume 83.4 fL, platelets 262,000/mL, prothrombin time 13.6 seconds (90.4% and INR 1.13). The signed informed written consent was obtained from guardian and from patient. The endoscopy was performed in the Endoscopy Service under general anesthesia using the endoscopic video imaging system Olympus GIF-Q150 (Japan), the biopsies were taken from the first and second duodenal portions and antrum. The duodenal aspirate was obtained using a sterile aspirate kit (Portex-Specimen-Trap, Smith Medical International) compatible with the suction mechanism in endoscope.

Duodenal tissue was placed in a glass container with 2% glutaraldehyde buffered in 0.1 M phosphate buffered solution at pH 7.2. Samples were submitted to the Experimental parasitology and Electron microscopy laboratories. The microscopic analyses of duodenal fluid, under phase contrast illumination showed *Giardia* trophozoites, subsequently, duodenal aspirate was cultured in sterile TYI-S-33 medium and incubated at 37°C. In a new stool sample from patient *Giardia* cyst were identify. Afterward, the patient was under treatment based on tinidazole at 75 mg/kg/day/4 days. Subsequent tests showed that lactose absorption was normal, stool examinations were negative for *Giardia* and abdominal pain had stopped.

Literature Search

A systematic review was made in PubMed (US National Library of Medicine National Institutes of Health) search database of invasive giardiasis in human. We searched the MEDLINE database for the following terms: “*Giardia* invasion” “*Giardia* invasion man”.

Electron Microscopy

The duodenal samples were post-fixed with osmium tetroxide, dehydrated in an ethanol series and embedded in epon resin. Semi-thin sections (0.5–1 µm) were cut and stained with toluidine blue for light microscopic examination. Ultra-thin sections (60–90 nm) were cut and collected on slot grids covered with formvar membrane. Sections were stained with uranyl acetate and lead citrate, and examined with a JEM-1011 (JEOL, Osaka, Japan) microscope.

Giardia Trophozoites Culture

The cysts from the stool samples were concentrated using a sucrose solution. The excystation was induced in Hank balanced salt solution (HBSS) at pH 2.0 for 30 minutes at 37°C. Induced cysts were washed with 37°C HBSS at pH 7.4, concentrated by centrifugation and placed in warm TYI-S-33 to complete excystation, as described elsewhere.³¹ Bacterial and/or fungal contamination was tested with cultures in blood agar and Sabouraud medium.

Genotyping

DNA samples from cysts and trophozoites were obtained using QIAamp spin columns following the manufacturer instructions (Quiagen GmbH, D-40724 Hilden, Germany). The *Giardia* isolate was genotyped using hemi-nested PCR and RFLP analysis of a segment from the glutamate dehydrogenase gene using primers described by Read⁹ and with the endonuclease *NlaIV*. The RFLP pattern was observed using 5% acrylamide gel electrophoresis stained with a silver stain kit (Bio-Rad Laboratories, Inc. 2000 Alfred Nobel Drive Hercules, CA 94547, USA) following the supplier instructions. The genotype was confirmed by repeating the PCR and RFLP analysis using reagents specific for a segment of the VSP417 gene.²⁵

RESULTS

Electron Microscopy

Electron microscopic analysis was negative for *H pylori*. The *Giardia* trophozoites were found in several areas. In semi-thin sections we found trophozoites in submucosa (Figure 1). Other trophozoites were found in the lumen as well as within the epithelium (Figure 2A–C). Electron microscopy showed trophozoites in tight contact with the intestinal microvilli (Figure 2A). Trophozoite with a typical crescent shape was also embedded in the tissue between the enterocytes nuclei and goblet cells. Scattered electron-dense granules were evident in the tissue, giving the impression that these granules were following the tracks of the parasites. The brush border appeared normal (Figure 2B). At higher magnification, the trophozoite had ultrastructural integrity it seems interacting with the enterocyte nucleus. There was cell lysis and damaged organelles surrounding the trophozoite (Figure 2B1). We found areas with either intact (BB) or abnormal (BB*) brush borders. In areas with abnormal microvilli, there were lysed cells and many electron-dense granules that formed a channel that appeared to flow into the tissue. Trophozoites were found between granules at the level of the nuclei and goblet cells (Figure 2C). At a higher magnification, we found that some trophozoites in the epithelium were structurally intact and other damaged. The presence of eosinophil sombrero vesicles (EoSVs) was observed³² (Figure 2C1, 2C2).

Giardia Culture and Molecular Genotyping

The *Giardia* trophozoites obtained by duodenal aspirate were unable to survive in axenic culture after 1 week. Trophozoites isolated by the excystation method were established in the axenic culture medium, and this *Giardia* isolate was designated INP220806-HGINV. The *Giardia* isolate belong to assemblage A, genetic group A2.

Literature Review

Reports of intraepithelial *Giardia* trophozoites are unusual; we found 5 reports in the literature.^{33–37} In these works, the tissue was processed with standard methods for light or electron microscopy and presents in clear way the trophozoites presence within the duodenal tissue (Table 1). Included the patient from this work 64 patients were analyzed. In 21 patients were seen trophozoites within epithelium, mucosa, lamina propria, submucosa or near of the crypts. The Sinelnikov³⁷ report is unclear because does not explain in how much of 14 patients they find with intraepithelial trophozoites. Besides, none was able to isolate and culture the *Giardia* trophozoites.

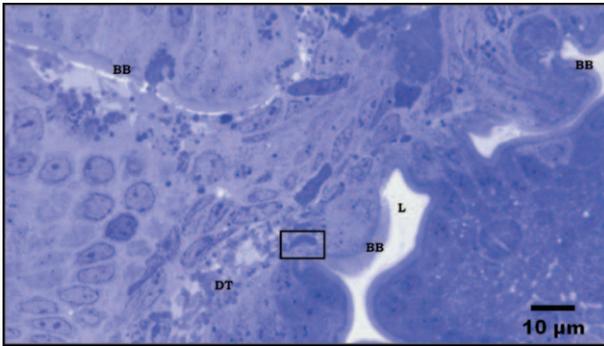


FIGURE 1. Semi-thin section from duodenal biopsy shows panoramic view of different cutting planes. Several areas of the epithelium with normal appearance and intact trophozoite within the damaged submucosa are shown. BB: Brush border; DT: Damage tissue. Stain toluidine blue.

Until now, this is the first culture of *G intestinalis* isolate with ability to invade the epithelium.

DISCUSSION

The intestinal invasion of *G intestinalis* trophozoites is not accepted.³⁸ Data from humans and experimental infections in animals show trophozoites in the lumen and on the surface of the mucosa of the small intestine. However, we found intraepithelial trophozoites in the biopsy from 1 patient with chronic abdominal pain and lactose malabsorption. For the first time *Giardia* trophozoites with potential to be invaders were successfully excysted, and maintained in culture in TYI-S-33 medium. Cyst and trophozoites from this isolate were genotyped; they belong to assemblage A, genetic group A2, which is a common genotype in our country.²⁵ These data are important because there are discrepancies regarding the use of assemblages/genotypes as pathogenic markers.²⁷⁻²⁹

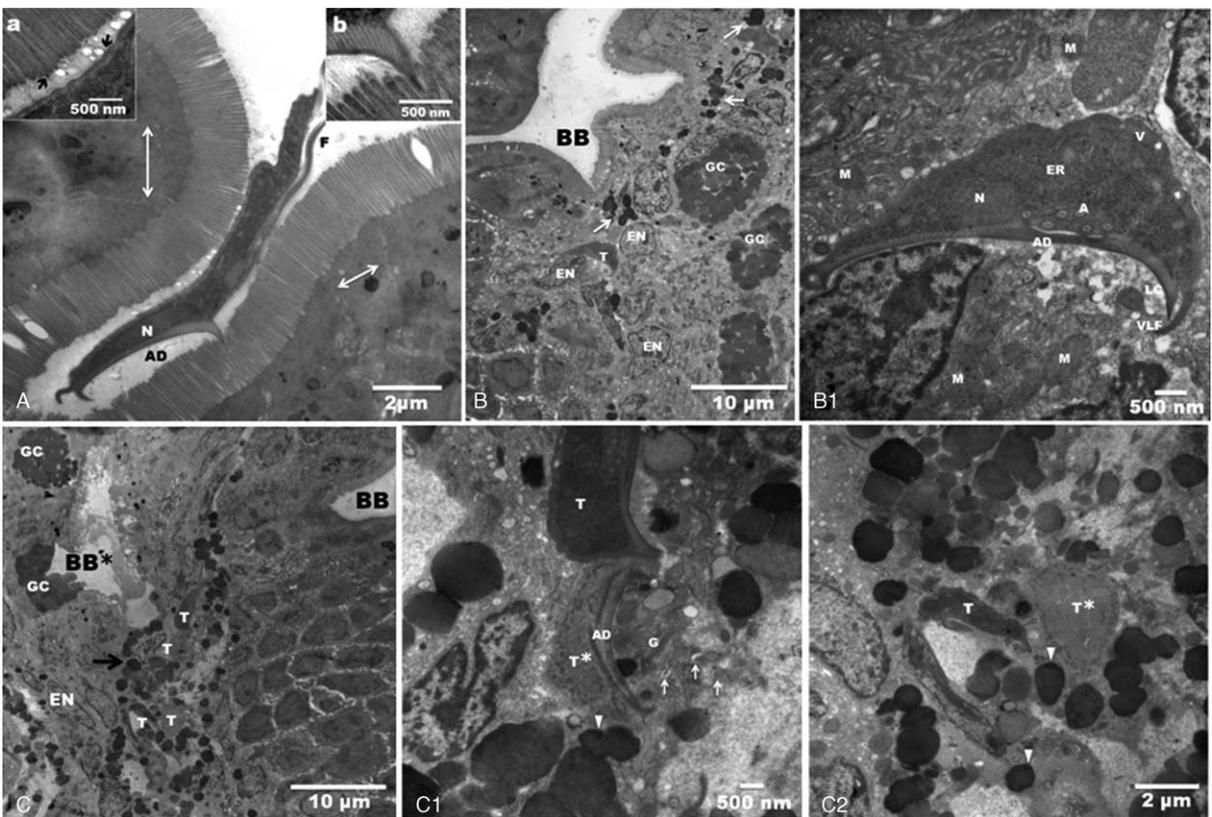


FIGURE 2. Transmission electron micrographs are showing intraepithelial trophozoites from a patient biopsy. A: *Giardia* trophozoite attached on the normal duodenal brush border, (N) nucleus, (AD) adhesive disc and (F) flagellum. There is a remarkable integrity of the intestinal intercellular tight junctions (arrows). Inset (a) shows small vacuoles between cellular microvillus and the plasma membrane of the trophozoite (arrows), suggesting a biochemical interaction. Inset (b) shows that the microvilli and ventral disc interactions give rise to an electron-dense zone. B: Panoramic of the duodenal epithelium with a brush border (BB) of normal appearance. At the level of the enterocyte nuclei (EN) and goblet cell (GC) there is a trophozoite (T). Note the electron-dense granules dispersed in the tissue (arrows). B1: Higher magnification shows the normal architecture of the trophozoite, which appears to be attached with its adhesive disc (AD) to one nucleus. Note the vacuoles (V) in the normal plasma membrane, endoplasmic reticulum (ER), axonemes (A), nucleus (N), lateral crest (LC), and ventrolateral flange (VLF). Lysed cells were found near the adhesive disc. Several damaged mitochondria (M). C: Low magnification of the duodenal epithelium. There is damage in the epithelial tissue and in the brush border (BB*), which is the likely site of entry of the trophozoites (T). We clearly see 4 trophozoites surrounded by electron-dense granules (arrow). In another area, the brush border appears intact (BB). C1: High magnification shows an intact trophozoite (T) and another trophozoite with total lysis of the dorsal membrane (T*), and fragmentation of its adhesive disc (AD). Nearby, there are eosinophil sombrero vesicles (arrows), granules (arrowheads), and Golgi bodies (G). C2: Two *Giardia* trophozoites surrounded by granules (arrowheads); one looks swollen (T*), and the other has a normal morphology (T).

TABLE 1. Invasive Giardiasis Summary of Reported Cases

Patients	Intraepithelial	Authors
Asymptomatic 10 patients HM	Six cases. Trophozoites penetrating the epithelium. Since mucosa to the submucosa. Trophozoites solitary and in groups. Normal villi. Without inflammation	Brandborg ³³
Asymptomatic male 64 year old, with steatorrhea HM, TEM	1 case. Normal mucosa intestinal. Trophozoites within the mucosa. Injury of the cells surrounding trophozoites. Without inflammatory reaction. Occasional eosinophils	Morecki & Parcker ³⁴
31 patients. 12 adults with steatorrhea and invasive trophozoites HM	12 cases. In 4 patients trophozoites were found in duodenum, 5 in jejunum and 3 in both. Trophozoites in process of entry, within the epithelium and lamina propria. In 7 patients: villi were short and broad, transmigration of the inflammatory cells, brush border was lost and goblet cell increased in number	Saha & Ghosh ³⁵
7 patients IM	1 case. Trophozoites in the lamina propria and near the crypts. Trophozoites within a damaged area	Fleck ³⁶
14 patients with giardiasis. c-kit	1 case. Trophozoites stained with c-kit immunohistochemical were seen in intraepithelial location	Sinelnikov ³⁷
Female pediatric patient with lactose malabsorption, abdominal pain TEM	1 case. Trophozoites with normal appearance within damaged epithelium and surrounding of granules. Trophozoites near the injury brush border	This study

HM = Histological Method, IM = Immunocytochemistry Method, TEM = Transmission electron microscopy.

The literature review showed 20 cases of invasive *Giardia* trophozoites, including this one there are 21 cases with invasive trophozoites.^{33–37} Previously we showed trophozoites during the invasion process and goblet cells hyperplasia in samples from dogs.²² Here, we are showing trophozoites with and without a normal morphology inside the epithelium, many granules surrounded the trophozoites, which may be from eosinophils because EoSvS were recognized. No inflammatory cells were observed. Owen et al³⁹ suggest that reports showing invasive trophozoites result from mistakes in the sample collection and preparation processes, rather than true invasion. Trophozoites may take advantage of breaks in the epithelium before fixation and may use their flagella to enter into the epithelium.

This and other studies evidence *Giardia* trophozoites inside the intestinal epithelium, suggesting that there is invasive *Giardia* isolates, but these trophozoites are not detected because the duodenal biopsy is an invasive technique and giardiasis diagnosis is performed by stool analysis. Future studies are necessary to reproduce, based on the INP220806-HGINV culture, the epithelial invasion phenomenon in an animal model, and to determine whether intraepithelial parasites play a role in the pathogenic mechanisms involved in symptomatic giardiasis.

In the medical literature there are few cases of invasive giardiasis. Analysis of this case and the review of the literature suggest that patients with steatorrhea, abdominal pain, or lactose malabsorption could have trophozoites with the potential to be invaders.

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