Demonstration of Trophozoites of *G. Lamblia* in Ileal Mucosal Biopsy Specimens May Reveal Giardiasis in Patients With Significantly Inflamed Parasite-free Duodenal Mucosa

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Abstract: In the majority of individuals, infestation with trophozoites of *Giardia lamblia* (synonymous *G. duodenalis* or *G. intestinalis*) leads to a self-limited disease. Whereas most duodenal biopsies with chronic giardiasis show little or no inflammatory reaction, some patients may develop a severe disease with significant mucosal inflammation and various degrees of villous blunting. Occasionally, the histologic changes may resemble those of celiac disease. In this paper, we describe 11 patients, 5 of them female, with chronic giardiasis and demonstrable *G. lamblia* in ileal biopsies. The median age was 45 years (35 to 62 y), with male patients being at least 10 years younger than female patients. All of the duodenal biopsies showed at least mild villous blunting (grading: mild, marked, or total). In the mucosa an increased number of plasma cells and lymphocytes was observed. Furthermore, varying numbers of granulocytes were found in the lamina propria and in the epithelial layer. In 1 case only, the number of intraepithelial lymphocytes was >40/100 epithelial cells thus mirroring the histologic picture of celiac disease with a flat mucosa (with negative celiac disease–specific serological findings). Interestingly enough, all mucosal biopsy specimens from the duodenum were parasite free. Therefore, giardiasis could only be revealed by the demonstration of trophozoites of *G. lamblia* in biopsy specimens from the terminal ileum, which had been taken simultaneously or several weeks later. In contrast to duodenal biopsies, the ileal mucosa appeared either normal or only mildly inflamed in this setting. All patients but 1 were symptomatic, with chronic diarrhea being the leading symptom. Symptoms resolved after antibiotic therapy. This study demonstrates that giardiasis may be associated with a significant duodenal pathology in biopsy specimens without discernible parasites. In the cases described here infestation with *G. lamblia* was only proven histologically by examination of mucosal biopsy specimens taken from the terminal ileum.

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Giardiasis, a noninvasive protozoal disease of the gastrointestinal tract, is caused by the protozoon *Giardia lamblia*. Infestation with this parasite is found worldwide,[1] with *G. lamblia* being among the most frequently isolated intestinal parasites in industrialized countries.[2–4] Fortunately, this is a self-limited disease in >85% of cases.[5–7] In the remainder a chronic infestation may develop. Owing to a lack of awareness of this entity, giardiasis may remain undetected for a long period of time.[8]

G. lamblia is transmitted feco-orally through the ingestion of cysts. The parasites excyst in the stomach, duodenum, and upper jejunum.[9] Two trophozoites are released from 1 cyst and subsequently colonize the mucosa of the small bowel. The trophozoites are thought to undergo encystation within the small intestinal lumen. After encystation they pass through the gut and are excreted in the feces.[3] The disease is diagnosed by either the detection of cysts in the feces or the demonstration of trophozoites in aspirates of duodenal fluid or in duodenal mucosal biopsy specimens. For a long period of time it was thought that trophozoites are already encysted in the terminal ileum and can therefore not be found histologically in biopsies taken from this region. However, this is not the case as demonstrated in an earlier study.[10] Furthermore, trophozoites are rarely observed on the mucosa of the gastric antrum[11] and in colonic biopsies.[12]

Interestingly enough, in 96% of the patients, who have not been able to eliminate these parasites spontaneously, no histologic changes can be detected in the duodenal mucosa. In the remainder, a local inflammatory response is induced, which may be accompanied by villous blunting.[12]

In the present paper we report on duodenal biopsy specimens showing signs of significant inflammation in the duodenal mucosa with varying degrees of villous blunting. In 1 case a celiac disease–like histology was observed. In all our reported cases, trophozoites of *G. lamblia* were not detectable in this part of the small bowel. The correct diagnosis could only be rendered after detection of parasites in mucosal biopsy specimens of the

terminal ileum, which had been taken simultaneously or in the follow-up ileocolonoscopy.

MATERIALS AND METHODS

Between 2005 and 2015, 11 cases were retrieved from our files showing an inflamed duodenal mucosa and varying degrees of villous blunting but no clearly visible trophozoites of *G. lamblia* in the duodenum. In all cases biopsies from the terminal ileum revealing colonization by these parasites were available. Seven of these cases were being observed throughout the last 3 years. For comparison, the number of patients signed out with the diagnosis "giardiasis" in duodenal biopsies between 2012 and 2015 was determined.

Histology

The degree of villous blunting was determined semiquantitatively: Cases with a villous length of less than one third of the expected one were classified as mild villous blunting. In cases with a flat mucosa no villi were observed (total villous atrophy). Specimens with marked villous blunting showed a villous length between mild villous blunting and flat mucosa. Parallel to the reporting scheme in celiac disease[13] the various degrees of villous blunting were given as 0 or I (normal architecture without or with an increase in IEL), IIIa (mild), IIIb (marked), and IIIc (total).

At least 300 epithelial cells (EC) were counted in the villi, and the number of intraepithelial lymphocytes (IELs) detected in between these ECs was given as number of IELs per 100 ECs.

Furthermore, it was stated whether the number of granulocytes and mononuclear cells was considered normal or increased (+). The degree of villous blunting was also determined in the terminal ileum. The number of parasites was determined semiquantitatively: When no more than 10 trophozoites were found in all specimens available the infestation was considered mild (+), when no more than 5 parasites were found in a high-power field the parasite density was graded as intermediate (+ +), cases with more than 5 trophozoites in a high-power field were considered severely colonized (+ + +).

RESULTS

Age and Sex

Five patients were female and 6 were male. The median age of the female patients was 59 years (range 55 to 62 y) and that of the male patients was 40.5 years (range 35 to 45 y). The overall median age was 45 years (range 35 to 62 y).

Duodenal Mucosa

A median of 3 biopsies was taken from the duodenum (range 2 to 16 biopsies). A significant degree of villous blunting was found in all patients but 1 (Table 1). In 7 cases varying degrees of villous blunting were observed in every biopsy; in 3 cases the mucosa showed a normal architecture in at least 1 biopsy. The specimens of the only asymptomatic case showed only slightly shortened villi.

TABLE 1. Patients

	Duodenum		Ileum		
Patient	**Stage**	**IEL**	**IEL**	**Inflammation**	***G. lamblia***
M, 35	0	12	8	Yes	+
M, 44	IIIa and c	10	12	No	+
F, 55	0, IIIc	8	8	No	+ + +
F, 61	IIIb and c	10	12	Yes	+ +
M, 38	IIIb and c	7	9	Yes	+ + +
M, 43	IIIa and b	7	12	Yes	+ +
M, 37	IIIb and c	10	42	No	+ + +
M, 45	0, IIIa	30	9	No	+
F, 62	IIIc	64	8	No	2
Follow-up	IIIa and b	62			
F, 59	0 and IIIc	9	32	Yes	+
F, 59	IIIa	12	42	Yes	+

The column "Patient" states sex and age; Stage: grade of villous atrophy of duodenal mucosa; IEL: number of IELs; Inflammation: states whether the ileal mucosa is inflamed (yes) or not (no); *G. lamblia*: the relative density of trophozoites is given as +, + +, or + + +.

Considering the most severe degree of villous blunting only, total villous atrophy was observed in 7 patients (Figs. 1–3) and marked (Fig. 4) and mild villous blunting in 1 and 2 patients, respectively. In 1 case no significant villous atrophy was observed. The overall median IEL count was 10 (range 7 to 64). More than 20 IELs were observed in 2 cases and >40 in 1 (Figs. 5–7). Granulocytes were found in all patients but 1 in the epithelial layer and in all patients in the lamina propria. The number of mononuclear cells was considered increased in all patients. In cases with villous atrophy, the epithelial layer showed epithelial flattening in the majority of cases.

In 1 patient the duodenal bulb was biopsied. It showed a normal villous architecture with a mild infiltration by mononuclear leukocytes and neutrophils. No trophozoites were observed in these specimens. All biopsies of the duodenal mucosa were free of parasites.

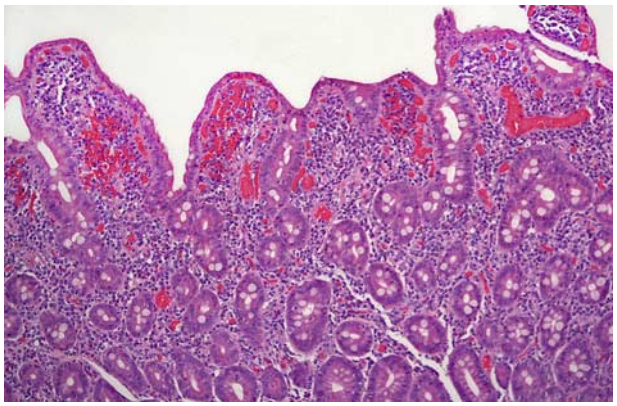

FIGURE 1. Duodenal biopsy with mostly total villous atrophy (IIIc); on the left side of the slide are very short remainders of the villi (IIIb). The surface epithelial layer is flattened. IEL numbers are not increased. Plasma cells are increased in number, along with neutrophils.

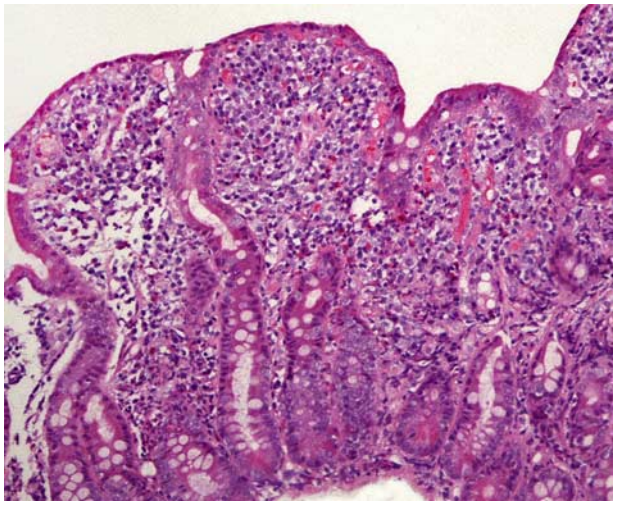

FIGURE 2. Flat duodenal mucosa (IIIc) with flattened surface epithelia, a normal number of IELs, lymphoplasmocytosis, and an increased infiltration of the lamina propria by neutrophils.

Ileum

A median of 3 biopsies was taken from the ileum (range 2 to 7 biopsies). In 2 cases mild villous blunting was observed; in the remainder the mucosal architecture was considered normal. A neutrophil infiltration of the epithelial layer and the lamina propria was found in 6 patients. Mononuclear cell content was considered normal in all patients. The overall median IEL count was 12 (range 8 to 42). More than 20 IELs were observed in 3 cases; >40 in 2. The number of parasites was given as +, ++, or +++ (Fig. 8) in 6, 2, and 3 cases, respectively.

In 1 case showing inflammation of the ileal mucosa (Fig. 9), infectious-type colitis was observed. In the ascending colon of this patient a few trophozoites were found (Fig. 10).

Between 2013 and 2015 some 122 cases with *G. lamblia* in duodenal biopsies were observed. During this period of time, duodenal biopsies taken from 35,263 individuals were submitted. This represents a detection rate of 1:295 in duodenal biopsies. In the same period of time, 9 cases with trophozoites of *G. lamblia* in the terminal ileum were observed. This represents a *Giardia* positivity rate of 1:2092 in the ileal biopsy specimens of the 18,831 individuals submitted.

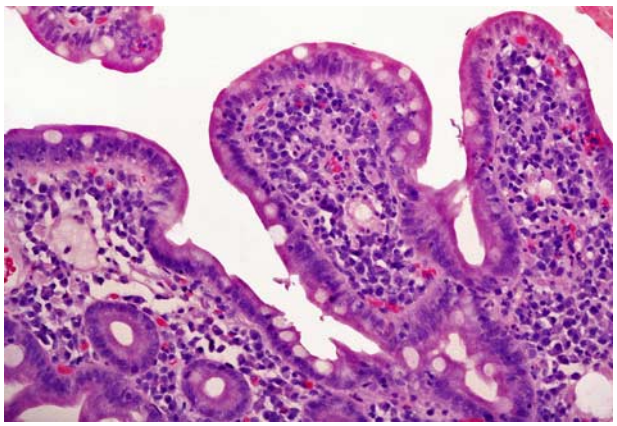

FIGURE 3. Duodenal mucosa with mild villous blunting (IIIa). The number of IELs is not increased. There is a moderate lymphoplasmocytosis and an increased infiltration of the lamina propria by neutrophils.

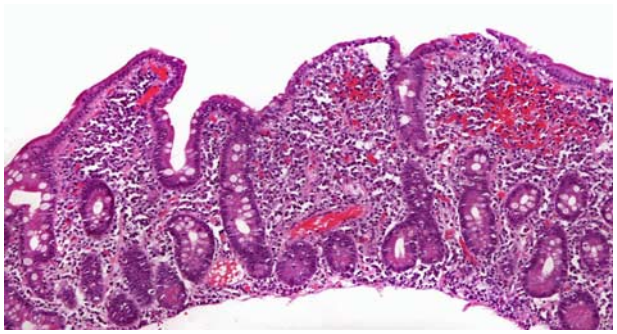

FIGURE 4. Duodenal mucosa with total villous atrophy on the right (IIIc) and marked villous atrophy on the left (IIIb). The number of IELs is not increased. There is a lymphoplasmocytosis and an increased infiltration of the lamina propria by neutrophils.

Clinical Findings

Ten patients were suffering from severe diarrhea for at least 4 weeks. In 2 patients it was considered watery. Some 4 patients lost weight, with a maximum of 8 kg in 1 of them. Nausea and vomiting was noted in 4 persons. One reported paraumbilical pain.

Only 1 individual was considered symptom free, undergoing opportunistic screening gastrointestinal endoscopy. No patient was considered immunocompromised.

Clinical Findings After Therapy

A follow-up was available in 10 patients. After therapy with either metronidazole (9 cases) or metronidazole and ciprofloxacin (1 patient), all patients were free of symptoms. In 1 case, follow-up biopsies were taken and were considered normal.

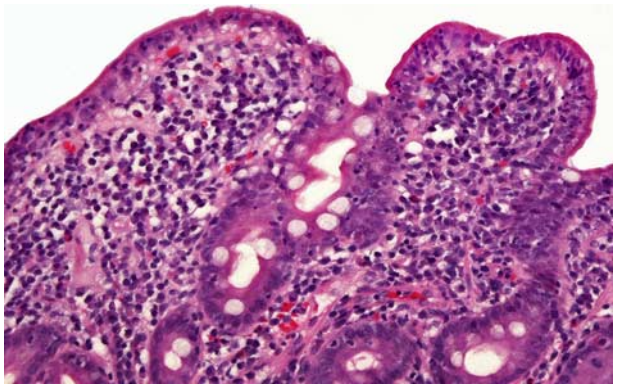

FIGURE 5. Flat duodenal mucosa (IIIc) with a significant increase in IELs and a moderate lymphoplasmocytosis, mirroring the picture of celiac disease.

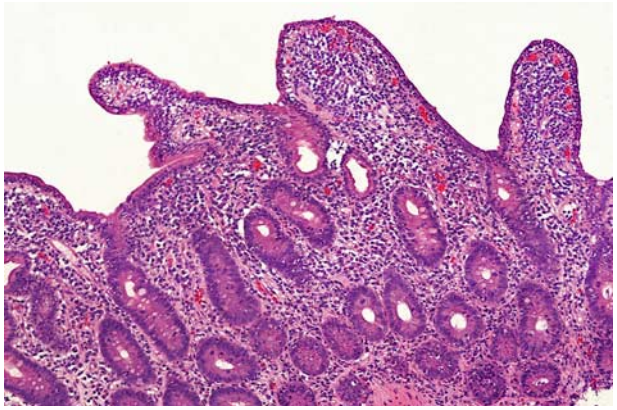

FIGURE 6. Second biopsy of the same patient with marked villous atrophy (IIIb) and a significant increase in IELs. There is a moderate lymphoplasmocytosis.

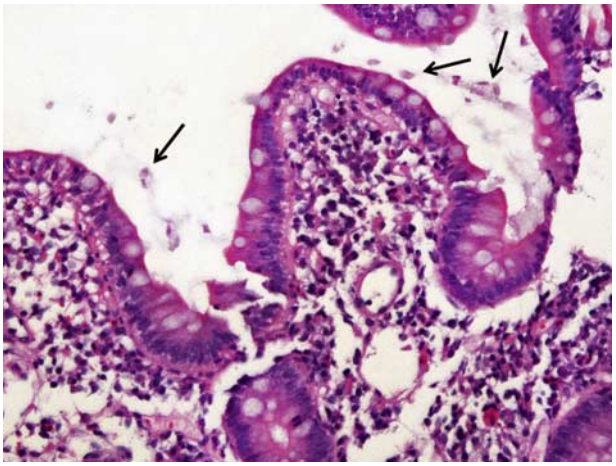

FIGURE 8. Only mildly inflamed ileal mucosa with numerous trophozoites of *G. lamblia* (arrows).

DISCUSSION

Particularly in patients with symptoms compatible with gastrointestinal diseases biopsies of the small bowel mucosa are collected to rule out a disorder of this part of the digestive tract. Mucosal biopsy specimens showing an inflamed mucosa in combination with villous blunting are diagnostically challenging as a number of underlying diseases have to be taken into account. In many of these cases a correct histopathologic diagnosis is of utmost importance for the revelation of the underlying disorder and subsequent appropriate therapy.

In this paper we want to draw the attention to an observation, which, to our knowledge, has not been described before. We report on 11 cases showing an inflamed yet parasite-free duodenal mucosa, mostly with significant villous blunting. Only biopsies taken from the terminal ileum, either simultaneously or in the follow-up, revealed an infestation with *G. lamblia*.

In 1 patient with a flat mucosa and a significantly increased number of IELs (64 IELs/100 ECs), we suggested celiac disease as the most probable differential diagnosis. However, serological studies (tissue transglutaminase antibodies [TTG]) did not corroborate our diagnosis. The follow-up ileal biopsies were considered to be normal. A rebiopsy of the duodenal mucosa revealed a comparable number of IELs but only mild to marked villous blunting still being compatible with celiac disease. The patient reported that she had been infested with parasites of *G. lamblia* in the past and that the current symptoms were similar to those experienced before. On reexamination of the biopsy specimens we were able to detect 2 trophozoites of *G. lamblia* in the ileal biopsy specimens (Fig. 7), whereas the duodenal mucosa was free of parasites. As a consequence, the histologic findings were considered compatible with chronic inflammation due to infestation with *G. lamblia*. The follow-up with an immediate resolution of the symptoms after antibiotic therapy corroborated this diagnosis.

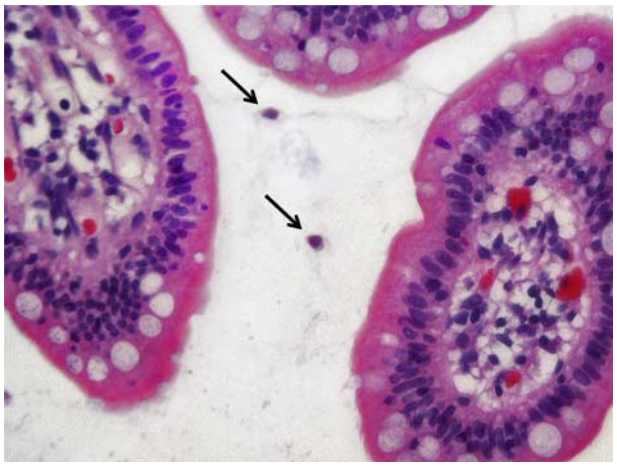

FIGURE 7. Normal-appearing ileal mucosa of the same patient with 2 trophozoites of *G. lamblia* (arrows).

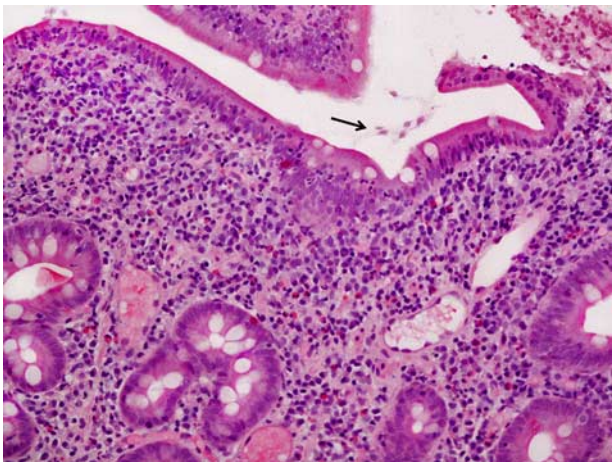

FIGURE 9. Inflamed ileal mucosa with severe colonization by trophozoites of *G. lamblia* (arrow).

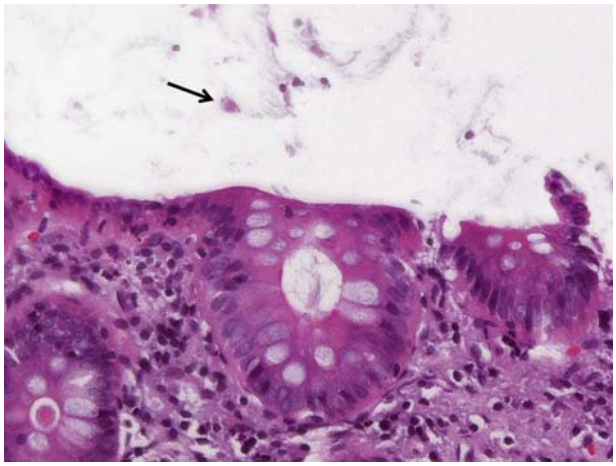

FIGURE 10. Colon biopsy specimen of the same patient with 1 trophozoite (arrow).

In fact, it is known from the literature that cases infested with *G. lamblia* may only rarely develop intestinal lesions similar to celiac disease. Furthermore, other disorders induced by a viral or bacterial infection of the small bowel,[14,15] tropical sprue,[16] the blind loop syndrome, intestinal bacterial overgrowth syndrome, or drug-associated enteropathy (eg, olmesartan)[17] may be associated with an identical mucosal morphology. Other rare disorders accompanied by a similar histology are autoimmune enteropathy[18] and immundeficiencies.[19] In Europe, however, celiac disease is by far the most frequent disorder showing a flat mucosa with an increase in IEL. Only in patients without TTG or antiendomysial antibodies (EMA) in the serum the above-mentioned differentials have to be considered.

In the remainder of the cases demonstrated in this paper, an inflamed mucosa with varying degrees of villous blunting from mild to total was observed. As the number of IELs was <40 IELs/100 ECs, infectious-type duodenitis was given as the most probable diagnosis. In 7 patients, trophozoites of *G. lamblia* were detected in the ileal mucosa specimens sent to us simultaneously with the duodenal biopsies. In the remainder the diagnosis was supported by the demonstration of trophozoites in the ileal mucosa taken at a later point of time.

The most important differentials in this situation, are, as mentioned above, chronic bacterial or viral infection or bacterial overgrowth. Furthermore, celiac disease may present with a number of IELs <40/100 ECs. However, we consider this diagnosis only in patients with positive serology (TTG, antiendomysial antibodies).[20] Other differentials such as Crohn disease or *Helicobacter pylori*–associated duodenitis in areas with gastric metaplasia can be ruled out due to the patchiness of the inflammation. Furthermore, endoscopic findings are different from those of celiac disease and chronic small bowel infection.

In the last 3 years, 122 cases with giardiasis were diagnosed in our institution, representing 0.29% of the submitted duodenal biopsy specimens. This is in concordance with earlier findings by one of our groups in another German laboratory.[12] In parallel to previous reports,[12,21] the duodenal biopsies not included in this study appeared free of any pathologic inflammation.

Although the number of female and male patients was comparable in our study, their age was not. Indeed, the median age of female patients was 18.5 years, higher than that of male patients, with the youngest female of this collective being 10 years older than the oldest male. This might be a bias due to the low number of cases studied. However, all female patients were older than 55 years suggesting that they were postmenopausal. Owing to the fact that the immunologic response is also modified by estrogen and progesterone, low levels of these hormones in postmenopausal individuals might contribute to the development of the histologic changes described in this paper. If this is the case, this fact would support the notion that also host-specific factors can play a role in the immunologic response to *G. lamblia*.

In an earlier paper we were first to describe that trophozoites of *G. lamblia* can also be observed in the ileal mucosa, supporting the importance of a search for these parasites in this part of the small bowel. Although, as shown in this report, biopsy specimens from the terminal ileum may reveal infestation with *G. lamblia*, this is not true for all patients. In the past 3 years we observed 2 individuals with trophozoites in the duodenal mucosa and a parasite-free ileal mucosa. In 1 case, the duodenal mucosa appeared inflamed; in the remainder and in the ileal biopsies the mucosa was considered normal (data not shown). As a consequence, it remains to be demonstrated whether suspected giardiasis should be demonstrated by ileal biopsy or an immunodiagnostic stool test.

Presently, it is not clear why the parasites could not be detected in the duodenal mucosa of our collective. Possibly, the significant inflammatory response may induce a decrease in the number of trophozoites, which may be so low that the parasites cannot be detected on histologic slides. Such a situation would parallel that in patients with lymphocytic gastritis elicited by *H. pylori*. In this histologic constellation the bacteria are only found in a minority of biopsy specimens, whereas in the majority of individuals the infection is only indirectly[22] recognized due to chronic active gastritis. Successful *H. pylori* eradication therapy results in the healing of the mucosa.[23]

As a conclusion, significant gastrointestinal symptoms such as severe diarrhea might be associated with an inflamed duodenal mucosa showing varying degrees of villous blunting. Recognizing an infestation by trophozoites of *G. lamblia* in this situation may be possible only in ileal biopsies as duodenal mucosal specimens may be free of parasites.

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