

Human intestinal spirochaetosis in Parma: a focus on a selected population during 2002-2005

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Abstract. *Background and aim of the work:* Human intestinal spirochaetosis (HIS) is a large bowel infection characterised by the colonization of the intestinal mucosa by spirochaetes belonging to the genus *Brachyspira*. The causative agents of HIS are *Brachyspira aalborgi* and *Brachyspira pilosicoli*. Symptoms of the infection, even if not specific, are long standing diarrhoea, abdominal pain, meteorism and rectal bleeding and sometimes they can suggest the clinical suspect of inflammatory bowel diseases or rectal carcinoma. Since poor data were available on the prevalence of this infection, the aim of our study was to describe the occurrence of this infection in our area in the period 2002-2005. *Methods:* During a period of 4 years we analysed 297 faecal samples from 99 patients selected by potential risk factors and symptomatology suspected for HIS. The diagnosis of HIS was performed by isolation and a molecular assay based on 16S rDNA restriction fragment length polymorphism (RFLP)-polymerase chain reaction (PCR). *Results:* From 2002 to 2005 we detected 12 cases of intestinal spirochaetosis, 7 caused by *Brachyspira aalborgi*, 4 by *Brachyspira pilosicoli* and one by both spirochaetes, which represented the first case of a mixed infection by 2 intestinal spirochaetes in our area. *Conclusions:* Despite the fact that HIS seems to be a low prevalence infection in our area, in a strongly selected population we found 12 cases of this infection (12.12%). These results stimulate us to extend the research of intestinal spirochaetosis in the general population, when long standing gastrointestinal disorders and potential risk factors are present. (www.actabiomedica.it)

Key words: Intestinal spirochaetosis, faecal samples, RFLP-PCR, prevalence

Introduction

The term “human intestinal spirochaetosis” (HIS) was used for the first time in 1967 to describe a large bowel infection, in which uncharacterised spirochaetes were found attached by one end to the colonic epithelium, forming a “false brush border” (1). At the electron microscopy, spiral-shaped microorganisms were observed on the luminal surface of the epithelium, end-on attached to the cell membrane in invaginated sites, between and parallel to shortened or destroyed microvilli (1).

Two anaerobic beta-haemolytic spirochaetes, *Brachyspira aalborgi* and *Brachyspira pilosicoli*, are the causative agents of human intestinal spirochaetosis (2-

5). *B. pilosicoli* is considered of zoonotic origin whilst *B. aalborgi* is restricted to humans and nonhuman primates (6, 7).

Clinical findings of HIS are various intestinal disorders, such as long standing and mucous diarrhoea, abdominal pain, rectal bleeding, weight loss and meteorism (8-11). Invasive colitis and cholestatic hepatitis were also diagnosed in patients with advanced infection by human immunodeficiency virus (HIV) (12) and appendiceal spirochaetosis was also rarely described in the literature, as an uncommon phenomenon, occurring with or without symptoms of acute appendicitis (13). *Brachyspira pilosicoli* has been also isolated from the blood of critically ill patients (14) and from blood cultures of an immunocompromised patient

who presented with fever, abdominal pain and bloody diarrhoea (15). Certainly all the symptoms described are not pathognomonic for the infection and in fact the clinical suspect of inflammatory bowel diseases or rectal carcinoma is often made (5, 8).

This infection has been described in immunodepressed patients, such as HIV infected subjects (12, 16, 17) or in patients with non-Hodgkin's lymphoma (15) but also in apparently healthy subjects with moderate gastrointestinal symptoms, thus it is not easy to identify certain risk factors for intestinal spirochaetosis.

In the literature it is described that after a correct diagnosis of intestinal spirochaetosis therapy with metronidazole is able to restore patients to health. It has also been demonstrated, comparing colonoscopy before and after the eradicating therapy in a patient with diagnosed intestinal spirochaetosis, that histological lesions were repaired with reconstruction of the damaged microvilli (8).

Little information is available on the distribution of human intestinal spirochaetosis in our area and the aim of our study was to describe its occurrence among a selected population of 99 patients who presented risk factors.

Materials and methods

During four years in our laboratory we analysed 297 faecal samples belonging to 99 patients of a selected population including 28 adopted children coming from developing countries, 7 HIV infected males with gastrointestinal disorders, and 64 patients who presented symptoms (long standing mucous diarrhoea, rectal bleeding, abdominal pain), potential risk factors (provenience from developing areas, precarious hygienic conditions), and other intestinal infections (detection of *Entamoeba histolytica* or other parasites in faecal samples) possibly related to intestinal spirochaetosis.

For the isolation of the spirochaetes two different selective media were used according to previously described methods (8): blood agar modified medium (BAM) supplemented with horse blood (7%) and trypticase soy agar (TSA) supplemented with sheep blood (5%). In order to obtain suspensions from faecal samples brain heart infusion (BHI) broth and trypti-

case soy broth (TSB) supplemented with foetal calf serum (FCS) 10% were used (8, 18, 19). Spectinomycin (S), rifampicin (R) and polymixin B (P) were added in combination to each media (BAM-SR and TSA-SP, respectively) and aliquots of faecal samples were streaked onto agar plates (8). The isolated spirochaetes were propagated in BHI and TSB supplemented with 10% FCS (8).

DNA of isolated spirochaetes was extracted using a "High Pure PCR Template Preparation Kit" (Roche), according to the manufacturer's instructions, and then a 16Sr DNA based on SER1 and SER2 primers was performed to amplify DNA (8).

Each amplification product was analysed by restriction fragment length polymorphism (RFLP), using restriction endonucleases *Hinf*I, *Sau*3AI, *Taq*I (Roche) and *Mbo*II (Amersham) and RFLP products were separated by electrophoresis in 4% agarose gel and stained with ethidium bromide (8).

Results

From July 2002 to December 2005 in our laboratory 297 faecal samples belonging to a selected population of 99 patients, hospitalised or outpatients, were analysed: 41 children and 58 adults, 27 females and 72 males, 47 Italians and 52 foreigners.

We detected 12 cases of HIS (12.12%), all diagnosed in males: 6 Italians and 6 foreigners, 4 children and 8 adults, as summarized in Table 1.

Infections were caused by *Brachyspira aalborgi* (7 cases), *Brachyspira pilosicoli* (4 cases) and one mixed infection by both spirochaetal species, *Brachyspira aalborgi* and *Brachyspira pilosicoli*. The patient with the mixed infection by *Brachyspira aalborgi* and *Brachyspira pilosicoli* was also infected by *E. histolytica*.

Among the 12 patients with HIS we also found that 7 patients had a concomitant intestinal parasitosis: 1 *Hymenolepis nana*, *Giardia intestinalis*, *Entamoeba dispar*, *Entamoeba coli*, *Blastocystis hominis*; 1 *Blastocystis hominis*; 1 *Entamoeba histolytica*; 1 *Giardia intestinalis*; 1 *Giardia intestinalis*, *Hymenolepis nana*; 2 *Entamoeba coli*, *Entamoeba dispar*, *Blastocystis hominis* (Table 1).

Clinical information was available for these pa-

Table 1. Cases of intestinal spirochaetosis during 4 years in Parma

Patient code	Sex	Age	Nationality	Intestinal spirochaetes detected	Intestinal pathogens/agents other than spirochaetes
1	male	49	Italian	<i>B. aalborgi</i>	None
2	male	36	Italian	<i>B. aalborgi</i> + <i>B. pilosicoli</i>	<i>Entamoeba histolytica</i>
3	male	75	Italian	<i>B. pilosicoli</i>	None
4	male	3	Foreigner	<i>B. aalborgi</i>	<i>G. intestinalis</i> + <i>H. nana</i>
5	male	1,5	Foreigner	<i>B. pilosicoli</i>	<i>G. intestinalis</i>
6	male	53	Foreigner	<i>B. aalborgi</i>	None
7	male	3	Foreigner	<i>B. pilosicoli</i>	None
8	male	40	Italian	<i>B. aalborgi</i>	<i>Entamoeba coli</i> + <i>B. hominis</i> + <i>E. dispar</i>
9	male	10	Foreigner	<i>B. aalborgi</i>	<i>Entamoeba coli</i> + <i>B. hominis</i> + <i>E. dispar</i>
10	male	36	Italian	<i>B. pilosicoli</i>	None
11	male	61	Italian	<i>B. aalborgi</i>	<i>B. hominis</i>
12	male	15	Foreigner	<i>B. aalborgi</i>	<i>G. intestinalis</i> + <i>H. nana</i> + <i>E. dispar</i> + <i>Entamoeba coli</i> + <i>B. hominis</i>

Table 2. Clinical information available for infected patients

Patient code	Signs and symptoms	Clinical data
1	Abdominal pain for almost one year	Not reported
2	Diarrhoea and abdominal pain	Not reported
3	Diarrhoea	Colonic polyps, <i>Helicobacter pylori</i> infection
4	Diarrhoea	Malnutrition
5	Diarrhoea	Intestinal malabsorption, reduced growth rate
6	Colitis	HIV infection
7	Not reported	Esophageal caustic stricture
8	Mucous diarrhoea, rectal tenesmus, inappetence, weight loss	HIV infection
9	Abdominal pain	<i>Situs viscerum inversus</i>
10	Mucous diarrhoea and fever	HIV infection
11	Bloody diarrhoea	Colonic polyps
12	Diarrhoea, abdominal pain, delayed growth	Not reported

tients and it is summarised in Table 2: 3 were HIV-infected, 2 had colonic polyps (one of them had *Helicobacter pylori* infection), 1 was affected by *situs viscerum inversus* (without clinical symptoms), 1 had reduced growth rate, 1 suffered from intestinal malabsorption and 1 from esophageal caustic stricture. No concomitant pathologies were described for the other 3 patients.

Conclusions

During a period of four years (2002-2005) 297 faecal samples collected from 99 selected people were sent to our laboratory for the research of intestinal spirochaetes. We detected 12 cases (12.12%) of infections

caused by *Brachyspira aalborgi* (7 patients), *Brachyspira pilosicoli* (4 patients) and both species (1 patient). Symptoms reported by the patients were long standing diarrhoea with mucous discharge, abdominal pain, meteorism, intestinal malabsorption, weight loss, and inappetence: since these symptoms are not specific for spirochaetal infection the first problem for the clinicians was to target a specific intestinal disease. In fact, in most of the cases the suspect of intestinal spirochaetosis was not made and other pathologies, such as inflammatory bowel diseases or rectal carcinoma were suspected for the patients and they were subjected to invasive investigations, such as colonoscopy.

It seems of great interest that 7 patients were also infected by intestinal parasites, thus we can hypothesize that intestinal spirochaetosis and parasitosis may have the same potential risk factors and ways of infection, particularly faecal contamination, as already postulated by other authors (6).

In particular, the patient with mixed infection by *Brachyspira aalborgi* and *Brachyspira pilosicoli* was also infected by *Entamoeba histolytica*. He suffered from long standing mucous and bloody diarrhoea with 5-6 daily discharges, meteorism, and abdominal pain, and was subjected to a colonoscopy, revealing the presence of ulcers on the mucosa of the rectum, sigmoid colon, and caecum and initially the clinician advanced the suspect of Crohn's disease (20). Moreover, in our study 2 infected patients were children adopted from developing countries where they lived in precarious hygienic conditions; they were also affected by intestinal parasitosis (one by *Giardia intestinalis* and *Hymenolepis nana* and the other only by *Giardia intestinalis*). One of them that was adopted from Bolivia presented malnutrition and signs of rickets, his age was only presuntive and estimated around 3 years old, while the other child (adopted from Ukraine) had intestinal malabsorption and delayed growth. Among the 12 infected patients we found 3 HIV-positive males: two were homosexual and we can suppose that the transmission of the intestinal spirochaetosis may be associated with sexual practices, as previously reported in the literature (21). The other patient was infected by the serotype 2 of HIV, at the B2 stage (22), and didn't suffer for intestinal disorders, but was subjected to clinical investigations because of a sideropenic anaemia not responsive to the therapy and supposed to be caused by an intestinal parasitosis.

Even though our results were referred to a restricted group of patients and we found that intestinal spirochaetosis has a significative prevalence in our study (12.12%), we did not have information on its occurrence among the general population. This fact stimulates us to research intestinal spirochaetes in all the cases characterised by vague and chronic gastrointestinal symptoms when a clinical suspect is not formulated. It would also be interesting to take into account intestinal spirochaetosis when making differential diagnosis with other intestinal pathologies, in par-

ticular inflammatory bowel diseases and rectal carcinoma.

As reported in the present study, the diagnosis of intestinal spirochaetosis can be promptly made using a PCR assay directly on the faecal samples and this is very relevant since the patients can avoid invasive investigations (such as colonoscopy). Moreover, it would be interesting to subject adopted children coming from developing countries to a complete screening for intestinal pathogenic agents including intestinal spirochaetes, since they come from areas with low hygienic conditions and are often simultaneously infected by intestinal parasites and since the therapy for the parasitosis does not resolve HIS causing a chronic infection. A targeted therapy for HIS is important in these children since HIS causes malabsorption and/or reduced growth rate when it becomes chronic (23).

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