THE USE OF QUINACRINE IN NITROIMIDAZOLE-RESISTANT *GIARDIA DUODENALIS*: AN OLD DRUG FOR AN EMERGING PROBLEM

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SUMMARY:

The study describes an almost 20% prevalence of imported refractory giardiasis diagnosed in 3 Tropical-Medicine Units in Barcelona, shows a 100% efficacy rate of quinacrine in nitroimidazole-refractory cases and compares genetic characteristics of *Giardia* cases.

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ABSTRACT

BACKGROUND: There is little evidence in the management of refractory giardiasis after treatment with nitroimidazoles. This study estimates the proportion of persistent giardiasis in 3 hospitals in Barcelona, describes risk factors and genotype associated and evaluates the efficacy rate of quinacrine in those with persistent giardiasis.

METHODS: A clinical prospective observational study was conducted in patients with giardiasis treated with nitroimidazoles. Those with persistent giardiasis were provided quinacrine. Molecular characterization of Giardia isolates was performed by PCR amplification of a fragment of *tpi* and *bg* genes.

RESULTS: Seventy-seven patients were recruited and treated with nitroimidazoles and in 14/71 of patients followed-up(20%), *Giardia* persisted. Refractory giardiasis was associated with malaise(p:0.007) and anorexia(p:0.019), with previous giardiasis(p:0.034) and with previous antibiotic(p:0.02) or antiparasitic(p:0.037). Quinacrine had an effectiveness rate of 100% in refractory giardiasis(n=13,95%CI 75-100).

Molecular characterization showed that 17(25%) *Giardia* isolates belonged to assemblage A, 31(43%) to assemblage B. In refractory giardiasis, assemblage B and A were found as responsible in 6 and 4 cases respectively.

CONCLUSIONS: Almost 20% of patients presented persistent giardiasis after nitroimidazole being both assemblage A and B involved. Short course of

quinacrine was effective in treating refractory cases and further controlled studies should evaluate its efficacy and safety.

INTRODUCTION

Giardia duodenalis is one of the most common intestinal parasitic protozoa reported in humans(1). The protozoon is distributed worldwide(2,3) and estimates indicate that more than billion people are at risk of giardiasis infection. It is much more prevalent in developing countries due to its association with poverty(4) where water contaminated with *G.duodenalis* cysts frequently causes travelers-related giardiasis(5). In industrialized countries, the infection is re-emerging(6) causing waterborne and to a lesser extent food-borne outbreaks(6,7).

Symptoms range from asymptomatic infection to mild and chronic diarrhea(8,9) The causes to explain the variability of symptoms remain unclear although multiples factors have been proposed. Some findings suggest that age and previous *Giardia* exposure are associated with mucosal inflammation and the intensity of symptoms(10). Geographic and population variation can also affect the host immune response against *G.duodenalis*(11). Genetic variability of *Giardia* strains has been also suggested to influence the simptomatology of the disease, although no association has been found between *Giardia* genotypes (assemblage A and B) and clinical symptoms when comparing different studies(1,12).

PCR-techniques , in addition to providing excellent sensitivity and specificity compared with microscopy and antigen detection methods(13–15), have being extremely useful in recent years to improve knowledge and understanding of

G.duodenalis genetics. Application of these techniques to characterize the *Giardia* isolates responsible for cases of refractory giardiasis has identified the assemblage B as responsible for these cases worldwide. To our knowledge, there have been no reports of refractory giardiasis produced by *Giardia* assemblage A. However, not all reported cases have been typed and the limited number of those studied makes it difficult to establish a definitive association between assemblage and resistant giardiasis.

Persistence of giardiasis despite nitroimidazole therapy is also relatively common in patients with chronic giardiasis accounting for about 20% of cases(16). This may be due to re-infection, immunosuppression or drug resistance(17). There is no standardized second-line regimen for refractory giardiasis. A second course of nitroimidazoles given for several consecutive days, and changing the type of antiparasitic agent and combination therapy have been tested in these cases(17). Quinacrine has been reported to have high efficacy-rate to treat giardiasis(1) although it has been associated with severe side-effects and poor adherence. In a previous retrospective study we demonstrated a 100% efficacy rate in patients treated with this drug using 7-day short course regimen, which was not associated with severe adverse effects(5).

This study aims to estimate the proportion of nitroimidazole-resistant *G.duodenalis* in the population attending three Tropical Medical Units and attempt to describe the risk factors and the assemblages associated with the persistence of giardiasis.

Further, the effectiveness of quinacrine in the treatment of these patients with resistant giardiasis is assessed.

METHODS

Study design, participants, and setting.

A prospective observational study was conducted in 3 specialized Tropical Diseases Units in Barcelona, Spain. From July-2012 to July-2013, all patients diagnosed with *G.duodenalis* attending the Tropical Medicine Unit (TMU) from Hospital Clinic, TMU-Drassanes and Hospital Sant Joan de Deu in Barcelona were invited to participate in the study. Patients unwilling or unable to give informed consent were excluded. For 95% of confidence level and a 20% of refractory giardiasis established by previous studies(16), considering a total of 71 patients, the final precision obtained was 10%. Participants were interviewed using a semistructured questionnaire. Particular emphasis was put on previous travel during the last 6 months, detecting previous gastrointestinal diseases, and other factors that could contribute to malabsorption. Gender, age, height (cm) and weight (kg) were recorded. All patients provided a stool sample before they were treated.

Standard formalin-ether concentration of all the stool samples was microscopically examined for the presence of *Giardia* cysts or trophozoites. In addition, an immunocromatography test was performed in fresh samples with the Stick *Giardia* test (Stick *Giardia* antigen kit, Operon SA). The

combined use of these two techniques aimed to achieve a greater sensitivity for those cases with low number of cysts and to minimize the possibility of obtaining false negatives. All patients were initially treated with nitroimidazoles (adults and children aged >5 years with tinidazole 2g, one day and children aged <5 years, with metronidazole 30mg/Kg/day, 7 days). Two control stool samples were collected and analyzed by microscopy and immunochromatography (IC) 30 days (+/-10 days) after treatment. Those infections with confirmed persistence of *G.duodenalis* (by detection of cysts in fecal samples or by IC were defined as refractory giardiasis and were subsequently treated with quinacrine at a dose of 100mg tid, 5 days. In children, the dose was adjusted to 8mg/Kg/day, 5 days.

Other routine tests such as stool cultures, microscopic observation or parasitic serologies were conducted to investigate the presence of other intestinal agents that may cause intestinal symptoms. HIV and IgA deficiency tests were performed in those patients diagnosed with refractory giardiasis.

Molecular characterization

An aliquot of all fecal samples was refrigerated and sent to the University of Zaragoza where further molecular analysis was undertaken. Previous to DNA extraction, a treatment of samples was performed in order to improve DNA quality. *Giardia* cysts were purified and concentrated using a sucrose gradient flotation method. Thereafter, cysts underwent 5 cycles of freezing (at -80 ° C, for

30 minutes) - defrosting (at 100 °C, for 10 minutes) and a final treatment with proteinase K (final concentration 100 µg/ml, overnight). When no cysts were observed, fresh stool samples were given the same pre-treatment with a sucrose gradient flotation method. DNA extraction was carried out after digestion of cysts or fresh samples using a commercial kit (Stool DNA Isolation Kit, Norgen Biotek Corp., Ontario, Canada) following the manufacturer's instructions.

G.duodenalis assemblage was determined by Polymerase Chain Reaction (PCR) of the triosephosphate isomerase (*tpi*) and ß-giardin (*bg*) genes following the protocols described by Sulaiman *et al.*(18) and Lalle *et al.*(19) respectively. PCR products were purified with GFXTM-PCR-DNA Gel Band Purification Kit (GE Healthcare) and then directly sequenced in both directions. The nucleotide sequences obtained were analyzed using BioEdit program (BioEdit sequence alignment editor, copyright @ 1997-2013 Tom Hall; Ibis Biosciences, Carlsbad,CA; URL:http://www.mbio.ncsu.edu/bioedit/bioedit/html) and they were compared with the sequences deposited in GenBank.

The DNA sequences obtained have been deposited in the Genetic sequence database at the National Center for Biotechnical Information (NCBI) under accession numbers JQ782391-JQ782407 and KX468980-Kx469069

Statistical methods

Data were double entered into an EpiData database; checked with EpiData software, version 3.1; and analyzed with STATA, version 13.

Statistical analysis

For numerical variables a normal distribution was tested with the Shapiro-Wilk normality test. As they did not follow a normal distribution, they were expressed as median (interquartile range –IQR) and were compared with unpaired Kruskal Wallis test.

Categorical variables were described with the number and percentages. They were compared using Monte Carlo and Fisher Exact test. To show the relation between patients with or without persistence of *G. duodenalis* and the rest of variables, OR and 95% confident interval were calculated

A backwards stepwise multivariate logistic regression analysis was performed to assess the variation of refractory giardiasis. Independent variables of the model were considered those that showed a $p<0.2$ with refractory giardiasis in the bivariate analysis. The goodness-of-fit of this model was calculated using means of the Hosmer-Lemeshow test, requiring a value of $p>0.05$. To avoid possible multicollinearity problems, it was required to have a variance inflation factor < 5 in all coefficients of the final model. The Odds Ratio with a confidence interval of 95% was used as the main estimator obtained from the statistical analyses(20).

Ethics: The study protocol and consent form were approved by the ethics committee of Hospital Clínic and Sant Joan de Deu Hospital and the ethic committee of Gol–i-Gurina.

RESULTS

General characteristics of the patients

Seventy seven patients were included in the study, 41(53%) were male and 22(29) were children (<18 years old). Twenty one(27%) were immigrants, 7(9%) were "visiting friends & relatives" (VFR), and 41 (53%) were tourist or worker travelers who had visited endemic countries. Eight patients (10%) had not travelled outside Spain in the previous six months. Patient characteristics by the geographic area where the infection was most probably acquired are summarized in Table 1. In almost half of the patients (37), *Giardia* was acquired in Asia (75% of these patients had visited the Indian subcontinent), 26% in Africa, 14% in Latin-America (LA) and 12% in Europe. Travelers were the most frequent group for giardiasis acquired in Asia (81%) whereas immigrants were more predominant for giardiasis from LA (55%) and from Africa (40%) and autochthonous patients (89%) in giardiasis acquired in Europe ($p<0.001$).

Most patients reported at least one risk factor for *Giardia* acquisition. Sixty-eight per cent of patients had consumed raw salad or raw meat and 60% of patients drank tap water in some occasions although this percentage was higher in people that had been in LA or Africa compared to Asia ($p< 0.001$). Tap water consumption during travel was also more frequent in migrants (82.35%) and VFR (85.71%) than travelers (55%),($p= 0.02$).

Around a third of patients had been working with patients (hospitals, day-care centres, nurseries…) and 4% had a previous episode of giardiasis.

Only eight patients (11%) were asymptomatic and this was more frequent in migrants (p= 0.018). Asymptomatic patients were more frequent in participants coming from LA, followed by Africa and Asia (p=0.009). In autochthonous infections, all patients were symptomatic. Having fever, abdominal pain, vomiting, weight loss or malaise were more frequent in patients coming from Asia (p= 0.004, p= 0.001, p= 0.005, p= 0.036, p= 0.01 respectively).

The following co-infections were observed: *E.histolytica/dispar* (5), *Ascaris lumbricoides*(2), *Strongyloides stercoralis*(3), *Trichuris trichiura*(1), *Blastocystis hominis*(3) *Schistosoma spp*.(2), *Cryptosporidium spp.*(1), and *Salmonella spp.*(1). However, persistence of giardiasis was not associated with having other concomitant intestinal parasitic infections. For each individual, we also calculated the number of risk factors for *Giardia* acquisition and the number of any other intestinal parasites. No statistically significant differences were found in the distribution of these variables in the geographic areas analyzed (see table 1)".

Refractory giardiasis

All patients were treated either with tinidazole, or in the case of children, metronidazole. Seventy-one patients had further followed-up after the initial treatment, and 20 of them (28%) were remained symptomatic. In 14 patients (20%; CI95% 11-29), *G.duodenalis* was found in the stool analysis. In the univariate analysis, the persistent giardiasis was more frequent when the infection was acquired in Asia (10/35, 29%) compared to Africa (3/18, 17%), America (1/11,

9%) or Europe (0/7, 0%) but this association was not statistically significant (p= 0.23).

Out of the three HIV patients, two were cured after tinidazole treatment and one was lost to follow-up. Refractory giardiasis was associated with systemic symptoms (malaise, p= 0.014 and anorexia, p= 0.049), and as expected, with previous antibiotic (p= 0.033). A relevant association was also found, although not statistically significant, between refractory giardiasis and having traveled to Asia (p=0.079), suffering diarrhea (0.090), having suffered previous giardiasis (p=0.094) and having been treated with antiparasitic (p= 0.097) (See Table 2).

Neither the initial treatment for giardiasis provided (metronidazole vs. tinidazole), the origin of *Giardia* acquisition, nor having severe symptoms was significantly associated with the persistence of *Giardia* in our model. According to the multivariate analysis, the risk of suffering refractory giardiasis is higher in patients with malaise (OR=5.02 CI=1.09-23.03), previous treatment (antiparasitic and/or antibiotic) (OR=6.19 CI=1.41-27.14) and in men (OR=2.85 CI=0.65-12.43) (See Table 3).

Following the study protocol, 13 patients with persistent giardiasis took quinacrine and tinidazole was prescribed for one child who had previously taken metronidazole. He was the only participant in whom *G.duodenalis* persisted despite the drug treatments, and clinically improved and was cured only after

receiving quinacrine in a third line course of treatment. Another child completed only 3 out of 5 days of quinacrine therapy due to adverse events, presenting irritability and somnolence that disappeared after stopping the treatment. However, *Giardia* was not found in subsequent serial stool examinations and he was considered cured.

Giardia analysis and characterization

Molecular analysis was conducted for 76 patients. A total of 105 sequences from 54 patients were obtained. Amplification was positive for 58 samples from 40 patients where *tpi* gene was analyzed and for 47 samples from 39 patients where the *bg* gene was analyzed. Amplification of the two genes simultaneously was positive for only 24 samples from 20 patients. The predominant genotype was assemblage B in 31 patients (42%) followed by A in 17 (25%) patients. A mixed infection by the two assemblages was observed in six patients and no amplification despite the observance of cysts was observed in 18 (25%). Negative PCR test was observed in 5 cases (7%).

Notably, out of 44 non refractory cases, 15 were PCR positive one month after the antiparasitic therapy (12 presented *Giardia* assemblage B, 2 assemblage A and 1 was a mixed infection A+B); although only three of them were still symptomatic. These non-refractory cases were followed during one month after treatment as planned.

For the 14 patients with persistence of *Giardia* cyst in stool examinations after nitroimidazole therapy (thirteen symptomatic and one asymptomatic), 6 of them presented *Giardia* assemblage B (5 Asia-acquired cases, 1 LA-acquired case). Three cases had an assemblage A (2 Africa-acquired cases, 1 Asia-acquired case) and in 4 cases there was no amplification and the PCR-test resulted negative. A specific assemblage was not associated with the persistence of *Giardia* ($p>0.05$). Homology of sequences of different samples of the same patient ranged between 97 to 100% for assemblage B (analyzed by amplification of *tpi* gene), and had 100% homology for those of assemblage A. When comparing sequences of *bg* gene, there were not any single nucleotide polymorphims (SNP). However, identity among sequences from different patients and different geographical locations was observed. One month after quinacrine therapy, all 14 patients improved clinically and fecal antigen detection and stool examinations were negative but PCR was still positive in 3 cases. Two of them were further followed up during two months and they were still positive for at least for 2 months after the quinacrine therapy. Both these patients belonged to assemblage B.

DISCUSSION

This work highlights an elevated proportion of refractory giardiasis after treatment with nitroimidazoles. It observed in almost 20% of patients treated with nitroimidazoles, which is similar to the reported efficacy of 80-90% elsewhere(17,21,22). Our study was not able to demonstrate any statistically

significant association with refractory giardiasis and the acquisition of giardiasis in Asia, as it has been reported elsewhere(16,23).

The differences in treatment failure among geographic areas could be attributable to acquired drug resistance, explained by several mechanisms. First, it has been proposed that that there is a geographical distribution of *Giardia* assemblages around the world although a particular assemblage of *G. duodenalis* has not been demonstrated to be associated with refractory giardiasis(24). In our study, assemblage B was predominant in refractory cases, consistent with other reports(25), although the study lacked the statistical power to demonstrate this effect. However, to our knowledge only cases of refractory giardiasis produced by *Giardia* belonging to assemblage B have been described. Therefore, this work is the first to describe refractory giardiasis by *Giardia* assemblage A, proving its plausibility and suggesting the importance of carrying out the molecular characterization of *Giardia* populations concerning refractory giardiasis.

Second, since the levels of drug pressure may be variable in different parts of the world where *G. duodenalis* is endemic, this could also be a key factor in the development of drug resistance in *Giardia* strains(26).

Being a traveler or a migrant was significantly associated with the geographic origin where the *Giardia* was acquired. Accordingly, for most travelers, *Giardia* was acquired when travelling to Asia, particularly to India, whereas for migrants, the geographic origin of *Giardia* was mainly either Africa or Latin-America. These findings could be explained because in the three sites where the study was carried

out, more than 40% of travelers go to Asia and migrants are predominantly coming from Africa and Latin-America (unpublished data).

Our data are in concordance with other published data of imported giardiasis in which the risk of giardiasis in returning traveler was higher in the Indian Subcontinent and Arab countries(27). Although our study could not demonstrate that giardiasis acquired in Asia is more likely to be refractory to first-line therapy, a greater proportion of refractory giardiasis was found in cases coming from this continent compared to the other regions.

Having severe symptoms such as malaise or anorexia before initial therapy, was associated with refractory giardiasis, and could be suggestive of higher parasitic-load infections. If this could be demonstrated, in these cases it could be considered to offer another treatment scheme, such as multiple-dose of nitroimidazoles(1).

The evaluation of treatment efficacy in *G.duodenalis* cases is rather complicated using the current diagnostic methods. PCR could be helpful to identify low parasitic load infections. However, our data suggest that almost 35% of patients presented positive PCR despite being considered cured according to the clinical and stool-tests findings; which is an unexpectedly high percentage compared with other studies that show a 100% of negativization of PCR one week after a 3-day course of metronidazole therapy(28). One possible explanation is that PCR sensitivity may be higher due to the molecular techniques undertaken for the genotypic characterization (in our study several PCR tests have been undertaken in the same sample what may have increased the global sensitivity). On the other

hand, as it has been reported by Martinez-Gordillo *et al*, the intraepithelial presence of a low number of *Giardia* trophozoites is possible, resulting in a positive PCR(29). Additionally, the variability obtained for sequences of tpi gene and the low variability of *bg* gene raises questions about what differences are there among *Giardia* assemblages and the usefulness of genotyping methods for their characterization. The significance of these results should be clarified in future studies.

Quinacrine demonstrated a 100% effectiveness rate, as has been previously described(5,30,31), and only one case of adverse events was observed which led to stop the treatment. This case was a child weighting less than 10Kg, making the optimal dose difficult to obtain due to a lack of pediatric formulation of quinacrine. However, even with a shorter 3-days regimen, quinacrine was effective to eradicate the parasite.

Thus, quinacrine should be considered as a second line drug treatment when available. Quinacrine is currently a medicinal product on a compassionate-use basis and therefore advocacy is needed to include giardiasis as one of its uses.

However, more controlled studies such as randomized trials are required to better evaluate the efficacy rate of quinacrine for giardiasis and particularly to monitor its potential side effects.

Besides the skin discoloration that has been reported, one of the main concerns when prescribing quinacrine is the occurrence of psychiatric adverse events

particularly in those people predisposed(32). In an study conducted during the Second World War in 7604 patients with malaria, quinacrine was shown to induce psychosis in 0.46% of patients(33). However, the mean total cumulative dose was 2100mg, which is higher than the 1500mg total dose recommended in our study. Moreover, the role of pre-existing malarial infection and previous combat experience that could have contributed to the clinical spectrum of the psychiatric disorders was not properly evaluated. Another prospective case series reported evidence of pronounced psychological stimulation in five cases after quinacrine administration with cumulative dose in the first day of 1200mg and a total cumulative dose higher than 4000mg; proving that quinacrine acts as a cortical stimulant(34). All patients had a rapid resolution of symptoms and there were no permanent sequelae noted. Finally, another more recent prospective study described neurologic and psychiatric adverse events in three patients with refractory giardiasis treated with quinacrine in combination with metronidazole, but again all patients were treated at a dose of 100mg t.i.d for 3 weeks which is equivalent to a cumulative dose of more than 6300mg(35) much higher than the dose recommended in our study. Another clinical trial conducted in children comparing mebendazole vs. quinacrine for the treatment of giardiasis, adverse events were frequently described in those treated with quinacrine. However, all adverse events were graded as mild, they did not affect the central nervous system nor warrant the discontinuation of the treatment in any patient(36).

In our study, all patients were cured with a 5-days schedule at a dose of 100mg tid. Only one of 14patients suffered an adverse event that caused discontinuation of the therapy. The patient was an 11-month-old baby that presented irritability and sleeplessness and it was not possible to evaluate the existence of a neuropsychiatric disorder. Further controlled studies are warranted to evaluate the efficacy and also the adverse effects of 3-5 days regimens of quinacrine.

CONCLUSIONS

Almost 20% of patients presented persistence of giardiasis despite nitroimidazole therapy. This outcome was not associated with a particular assemblage, as *Giardia* assemblage B was predominant, but some cases produced by *Giardia* assemblage A were observed. Short-course of quinacrine had 100% effectiveness although the optimal dosage remains unknown and the drug has not been approved to be used in giardiasis. Randomized controlled trials are needed to evaluate efficacy and safety of quinacrine as a second line therapy. Finally, PCR may persist positive in asymptomatic patients apparently cured according to clinical and stool test criteria.

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Table 1. Participants characteristics by subgroup

	Continent				p-value	Total (77)
	America (11)	**Asia (37)**	**Africa (20)**	**Europe (9)**		
Sex (male)	5 (46)	17 (46)	13 (65)	6 (67)	0.4	41 (53)
Age						
0-18	2(18)	4(11)	9(45)	7(88)	**<0.001**	22(29)
19-34	6(55)	18(49)	3(15)	1(13)		28(37)
35 maximum	3(27)	15(41)	8(40)	0(0)		26(34)
Patients						
Migrants	6(545)	6(16)	8(40)	1(11)	**<0.001**	21(27)
Travelers	4(36)	30(81)	7(35)	0(0)		41(53)
VFR*	1(9)	1(3)	5(25)	0(0)		7(9)
No travel	0(0)	0(0)	0(0)	8(89)		8(10)
Risk factors for Giardiasis	10 (100)	24 (67)	10 (59)	4 (57)	0.121	48
Unwashed raw food	9 (90)	19 (53)	14 (82)	0 (0)	**<0.001**	(69)
Unsafe drinking water	3 (30)	15 (42)	9 (47)	6 (86)		42 (60)
Working-care of patients.	1 (10)	1 (3)	0 (0)	1 (13)	0.124	33 (46)
Previous giardiasis	0 (0)	1 (3)	0(0)	0(0)	0.374	3 (4)
Sexual contact with *Giardia* (anal sex)	1 (9.09)	0 (0)	0 (0)	1 (11)	0.778	1 (1)
Previous intestinal disease					0.115	2 (3)
Symptoms associated with Giardiasis						
Asymptomatic	4 (36)	1 (3)	3 (15)	0 (0)	**0.009**	8(11)
Diarrhea	5(45)	28 (76)	14(70)	8 (89)	0.149	55 (71)
Fever	0 (0)	13(35)	1(5)	0 (0)	**0.004**	14 (18)
Abdominal pain	6 (55)	30 (83)	11 (55)	1 (13)	**0.001**	48 (64)
Vomiting	2 (18)	20 (54)	3(15)	1 (13)	**0.005**	26(34)
Aerophagia	4(36)	26 (70)	11 (8)	3 (38)	0.122	
Weight loss	3 (27)	25 (69)	7 (41)	3 (38)	**0.036**	
Malaise	4(36)	24(65)	6 (30)	1 (13)	**0.01**	
Anorexia	0 (0)	5(14)	0 (0)	0 (0)	0.130	

						44 (59)
*. VFR: Visiting friends and relatives. Proportions are expressed as cases/total number of patients (percent). Numerical values are expressed as median (interquartile range –IQR). Continuous variables were analyzed by using an independent H Kruskal Wallis test, and categorical variables were analyzed with Monte Carlo and Fisher Exact test.						38 (53) 35(46) 5 (7)
Previous treatments Antiparasitic Antibiotic Antidiarrhoeal	 0 (0) 0 (0) 1 (9)	 1 (3) 9(24) 4 (11)	 0 (0) 3 (15) 0 (0)	 1 (13) 0 (0) 1 (13)	 0.275 0.133 0.493	 2 (3) 12(16) 6 (8)
Other intestinal parasites *E. histolytica/dispar* *Ascaris lumbricoides* *Strongyloides stercoralis* *Trichuris trichiura* *Blastocystis hominis* *Schistosoma* spp. *Cryptosporidium* spp. *Salmonella* spp.	0(0) 0(0) 3 (27) 0(0) 2 (18) 0(0) 0(0) 0(0)	3 (8) 0(0) 0(0) 0(0) 0(0) 0(0) 1 (3) 1 (3)	2 (10) 2 (10) 0 (0) 1 (5) 0(0) 2 (10) 0(0) 0(0)	0(0) 0(0) 0(0) 0(0) 1 (13) 0(0) 0(0) 0(0)	----	5 (6) 2 (3) 3 (4) 1 (1) 3 (4) 2 (3) 1 (1) 1 (1)
HIV	1 (9)	0 (0)	1 (6)	1 (13)	0.377	3 (5)
IgA immunodeficiency	---	0 (0)	0 (0)	1 (100)	**0.018**	1 (12.5)
Number of risk factors: Median (IQR)	2.5 (1)	2 (1)	2 (2.75)	2 (2)	**0.142**	---
Number of other intestinal parasites: Median (IQR)	0 (4)	0 (0)	0 (0)	0 (0)	**0.141**	

Table 2. Factors associated with refractory giardiasis

	Refractory giardiasis Nº (%)	**Non refractory giardiasis Nº (%)**	**OR (CI)**	**p-value**
Gender				
Male	8/14 (57)	29/57 (51)	1.28 (0.40-4.18)	0.770
Female	6 /14 (43)	28/57 (49)		
Age				
<18 years	3/14 (21)	18/57 (32)	0.59 (0.16-2.24)	0.553
>18 years	11/14 (79)	39/57 (68)		
Patients type				
Migrant	3/14 (21)	15/57 (26)	0.76 (0.20-2.94)	1
Travelers	11/14 (79)	29/57 (51)		0.185
VFR*	0 (0)	7/57 (12)	3.54 (0.95-12.99)	---
No travel	0 (0)	6/57 (11)	---	---

Continent				
America	1 /14 (7)	10 /57 (18)	0.78 (0.21-3.01)	0.680
Asia	10 /14 (71)	25 /57 (44)		**0.079**
Africa	3/14 (21)	15/57 (26)	3.2 (0.94-10.80)	1
Europe	0 /14 (0)	7/57 (12)	0.76 (0.20-2.94)	---

Risk factors for Giardiasis	4/12 (33)	41/53 (77)	0.15 (0.04-0.57)	**0.005**
Raw food	7/12 (58)	32/54 (59)		1
Tap-water	4 /14 (29)	28/54 (52)	0.96 (0.28-3.25)	0.143
Working-care of patients.	2/13 (15)	1/54 (2)		**0.094**
	0/14 (0)	1/57 (2)	0.37 (0.1-1.33)	---
Previous giardiasis	0/14 (0)	2/57 (4)		---
Sexual contact with *Giardia*			9.63 (0.82-115)	

Previous Intestinal disease			---	

Symptoms of *Giardia*				
Asymptomatic	0/14 (0)	7/57 (12)	---	---
Diarrhea	13/14 (93)	39/57 (68)	6 (0.73-49.5)	**0.090**
Fever	4/14 (29)	10/57 (18)	1.88 (0.49-7.22)	0.453
Abdominal pain	9/13 (69)	36/57 (63)	1.31 (0.36-4.8)	0.599
Vomiting	4/14 (29)	21/57 (37)	0.69 (0.19-2.46)	0.757
Weight loss	8/13 (62)	28/55 (51)	1.54 (0.45-5.1)	0.555
Malaise	11/14 (79)	22/57 (39)	5.83 (1.46-23.3)	**0.014**
Anorexia	3/14 (21)	2/57 (4)	7.45 (1.12-50.3)	**0.049**
Previous treatment				
Antiparasitic	2/14 (14)	1/57 (2)	9.33 (0.78-111.5)	**0.097**
Antibiotic	5/14 (36)	6/57 (11)	4.7 (1.19-18.81)	**0.033**
Assemblage				
A	3/14 (21)	13/49 (27)	0.76 (0.20-2.97)	1
AB	0/14 (0)	1/49 (0)	---	---
B	6/14 (43)	23/49 (47)	---	1
Not typable	4/14 (29)	13/49 (27)	0.85 (0.27-2.72)	1
			1.11 (0.31-4.00)	

*VFR: Visiting friends and relatives. Proportions are expressed as cases/total number of patients (percent). Categorical variables were analyzed with Monte Carlo and Fisher Exact test. CI of OR were calculated by Woolf´s method

Table 3. Multivariate logistic regression model of the independent association of various variables with refractory giardiasis

	OR	CI	p-value
Sex: Man	2.85	**0.65-12.43**	**0.063**
Malaise: Yes	5.02	1.09-23.03	0.038
Previous tto: Yes	6.19	1.41-27.14	0.016

Test Hosmer Lemeshow= 1.179, p =0.758; R2 Nagelkerke = 0.294

FIGURE LEGENDS

Figure 1. Diagram about the clinical and treatment outcome of *Giardia* cases.