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Associations of *Giardia lamblia* assemblages with HIV infections and symptomatology: HIV virus and assemblage B were they born to each other?

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ABSTRACT

Giardia lamblia is an intestinal parasite that has an extensive genetic variation among isolates. This species is divided into eight different assemblages (A–H), but only assemblages A and B have been associated with human infections. Studies on the associations of *G. lamblia* assemblages and symptoms have been done but were inconclusive. The aim of this study was to correlate *G. lamblia* assemblages with symptoms in patients with and without HIV/AIDS and its association with the CD4 T cell count. The cross-sectional survey was conducted among patients attending the Evandro Chagas National Institute of Infectious Diseases (INI/FIOCRUZ) in Rio de Janeiro from January 2011 to February 2015. Thirty-eight of 65 microscopically positive stool samples for *G. lamblia* were from HIV positive patients and 27 were from HIV negative patients. Of the HIV infected patients, 19 (55.9%) were genotyped as assemblage B of which 9 (47.4%) had a CD4 T cell count below 200 cells/mm^3. In addition, we found a greater number of samples belonging to assemblage B in symptomatic cases (11 of 19; 57.9%). Our data suggest that assemblage B is very likely to be found in HIV infected patients and probably the lower CD4 T count gives advantages for assemblage B replication. Furthermore, assemblage B seems to be associated with symptomatology, particularly abdominal pain, asthenia, diarrhea, fever, headache and myalgia. This study provides information on *G. lamblia* assemblages and symptoms in patients with and without HIV/AIDS virus and their association with CD4 T cell counts.

1. Introduction

Giardia lamblia (syn. *G. intestinalis* and *G. duodenalis*) is considered one of the leading causative agents of diarrhea and is seen as an important waterborne disease pathogen that infects animals and humans worldwide (Feng and Xiao, 2011). The spectrum of clinical manifestations of giardiasis is quite variable, ranging from asymptomatic infections to acute or chronic diarrhea. The clinical manifestations of giardiasis are self-limiting in most cases, with transient intestinal complications that are usually solved completely, but because of the potential for chronic or intermittent symptoms, treatment is recommended (Eckmann, 2003; Robertson et al., 2010). The clinical signs of infection include diarrhea, bloating, vomiting, dehydration, abdominal pain, flatulence, and nausea. There is no appearance of blood in the stool since *Giardia* is a non-invasive parasite and few virulence factors have been identified (Ankarklev et al., 2010; Eckmann, 2003; Read et al., 2002).

A considerable amount of data has shown that *G. lamblia* is a species complex whose members show little variation in their morphology but present a remarkable genetic variability (Cacciò and Ryan, 2008; Thompson, 2004). Due to its invariant morphology, investigations of aspects such as biology, host specificity and transmission patterns require the direct genetic characterization of parasites from fecal samples. Actually *G. lamblia* is divided into at least eight distinct genetic assemblages (A-H), however only assemblages A and B are known to infect humans. The others are likely to be host specific, as assemblages C and D occur mostly in dogs and other canids, assemblage E in hoofed livestock, assemblage F in cats, assemblage G in rats and assemblage H in marine mammals (Lasek-Nesselquist et al., 2010; Monis et al., 1999; Thompson, 2004).

G. lamblia presents a heterogeneous clinical manifestations and one hypothesis is that the parasite assemblages could play a part in the development of symptoms. Molecular analyses have suggested that the genomic differences between assemblages A and B are sufficient to classify them into two different species (Adam et al., 2013; Franzén et al., 2009). Some authors believe that the genomic differences between strains WB (assemblage A) and GS (assemblage B) may explain some of the phenotypic differences (Jerlström-Hultqvist et al., 2010; Xu

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et al., 2012). Studies trying to associate *G. lamblia* assemblages with symptoms had been done all over the world (Haque et al., 2005; Minetti et al., 2015; Puebla et al., 2014). However, results from these studies have been controversial, with some authors correlating symptoms with assemblage A (Breathnach et al., 2010; Haque et al., 2005; Pestechian et al., 2014; Read et al., 2002; Sahagún et al., 2008), others with assemblage B (Gelanew et al., 2007; Homan and Mank, 2001; Lebbad et al., 2011; Minetti et al., 2015; Puebla et al., 2014), while others did not find any correlation (Kohli et al., 2008; Lebbad et al., 2008; Pelayo et al., 2008). Additionally, there have been very few studies about the correlations of *G. lamblia* assemblages with clinical manifestation in patients with the human-acquired immunodeficiency virus (HIV), and, again, conclusive results were not achieved (Lim et al., 2011; Maikai et al., 2012).

Immunocompromised patients are one of the high-risk groups for infection. Several studies have reported that individuals with immune deficiencies such as HIV/AIDS were more likely to have *Giardia* infection (Adamu et al., 2013; Boaitey et al., 2012; Sanyaolu et al., 2011). Indeed, patients with hypogammaglobinemia have been linked to a predisposition for chronic giardiasis (Oksenhendler et al., 2008). Hence, the aim of this study was to correlate *G. lamblia* assemblages with symptoms in patients with and without HIV/AIDS and its association with the CD4 T cell count.

2. Materials and methods

2.1. Population study

A total of 65 fecal samples positive to *G. lamblia* were collected between January 2011 and February 2015 from patients attending the Evandro Chagas National Institute of Infectious Diseases (INI/FIOCRUZ), a referral hospital in infectious diseases in Brazil, located in Rio de Janeiro. This hospital receives patients from all municipalities, mainly the metropolitan area. According to the last census conducted in 2010, Rio de Janeiro municipality had a population of 6,320,446 inhabitants and the metropolitan region (which is composed of 21 municipalities and is the second largest metropolitan area in Brazil) had 11,812,482 inhabitants (IBGE, 2010).

2.2. Data collection and laboratory procedures

Stool samples were collected by the patient in plastic disposable flasks without preservatives and maintained at 4 °C until laboratory analysis on the same day. The flasks were labeled with the name, collection date and the hospital number. The parasitological tests were conducted at the Parasitology Laboratory of INI by experienced laboratory technologists. This laboratory is certified by the College of American Pathologists. For laboratory diagnosis of *G. lamblia*, the fresh specimens were analyzed by means of centrifugation sedimentation (de Carli, 2001) and centrifugal flotation in zinc sulphate solution (Faust et al., 1938). The slides were then observed under the microscope (Nikon Eclipse E200, magnification of 10 and 40×).

In addition to parasitological tests, clinical and epidemiological data (age, educational level, gender and residence place) were collected from the hospitaĺs database. The patients were classified as asymptomatic or symptomatic (abdominal pain, asthenia, cough, diarrhea, fever, headache, myalgia, rapid weight loss, vertigo and/or vomiting). According to World Health Organization (WHO) criteria diarrhea is defined as the passage of three or more unformed stools per day or more frequent passage than is normal for the individual (WHO, 2013).

The hematological, biochemical (lipidogramme and proteinogramme) and/or immunologic results were collected from the patienťs clinical records, as well as the information about the CD4 T cells counts (cells/mm^3) and the adherence (or not) to the antiretroviral therapy (ART) in the HIV infected patients.

All patients attending INI/FIOCRUZ are dewormed when diagnosed and HIV infected patients received antiretroviral therapy (ART) according to Brazilian Ministry of Health's consensus recommendations (drugs are provided by the institution itself).

2.3. Genotyping of G. lamblia

DNA extraction was performed using the QIAamp DNA Stool mini Kit (Qiagen, Germany) according to the instructions of the manufacturer. The parasite assemblage and sub-assemblage were determined using PCR-RFLP amplification, and real time quantitative PCR (qPCR) (Faria et al., 2016).

2.4. Statistical analysis

The data entry was carried out using Excel software and analyzed using Statistical Package for the Social Sciences (SPSS) version 16. Percentages were used to perform the exploratory analysis of the categorical variables and quantitative variables are presented as mean ± standard deviation (SD). Pearson's chi-squared and Fisher's Exact Test were used for categorical data. The level of statistical significance was set as $p < 0.05$.

2.5. Ethical considerations

The Research Ethics Committee Evandro Chagas National Institute of Infectious Diseases (INI/FIOCRUZ) approved the study (protocol number: 127.542). This project was in accordance with the Brazilian Ethical Resolutions, especially Resolution CNS 196/1996 and its complementary and the Code of Medical Ethics of 1988 (articles 122–1307). Written informed consent was obtained from all patients or legal guardians of patients younger than 18 years, prior to sample collection. The informed consent was provided after a detailed explanation of the objectives of the work. A term of privacy and confidentiality was signed by the researches for patients for whom it was not possible to obtain informed consent beforehand.

3. Results

3.1. Characteristics of the study population

Among the 65 patients positive for *G. lamblia*, the majority were adults (69.2%) with an average of 32.54 ± 13.69 years (Mean ± SD; median = 32) (Table 1). The highest prevalence occurred between 30 and 39 years (33.3%) and there were more male than female patients (69.2% *versus* 30.8%). Seventy-two percent of patients were literate; most of them (53.8%) live in Rio de Janeiro municipality and 83.1% live in metropolitan region (Table 1).

Of these positive patients for *G. lamblia*, 38 were HIV infected patients and 27 were non-HIV-infected patients. In HIV infected patients, 14 (36.8%) had CD4 T cells counts higher than 500 cells/mm^3, 2 (5.3%) had between 350 and 500 cells/mm^3, 6 (15.8%) had between 200 and 350 cells/mm^3, and 16 (42.1%) had less than 200 cells/mm^3. As aforementioned all HIV infected patients were included in the ART programs, however, only 24 (63.2%) adhere to the therapy.

3.2. Patients clinical status and presence of co-infections

Evandro Chagas National Institute of Infectious Diseases attended individuals with HIV/AIDS, HTLV, sexually transmitted diseases (STDs), Chagas disease, toxoplasmosis, leishmaniasis, mycoses, tuberculosis, and acute febrile diseases (dengue, malaria, influenza, chicken pox, leptospirosis, among others). Of the 65 patients positive for *G. lamblia*, 21 (32.3%) showed clinical symptoms while 44 (67.7%) were asymptomatic (Tables 2 and S1). The majority of symptomatic patients were HIV positive (n = 16, p = 0.045), being diarrhea the most

Table 1
Characteristics of the patients infected with *Giardia lamblia* according to HIV status.

Characteristics	Overall (n = 65)		HIV+ (n = 38; 58.5%)		HIV- (n = 27; 41.5%)	
	No.	%	No.	%	No.	%
Gender						
Female	20	30.8	9	23.7	11	40.7
Male	45	69.2	29	76.3	16	59.3
Age group (years)						
0–14	6	9.2	1	2.6	5	18.5
15–25	12	18.5	6	15.8	6	22.2
26–65	45	69.2	31	81.6	14	51.9
Missing	2	3.1	–	–	2	7.4
Educational status						
Elementary school	17	26.2	12	31.6	5	18.5
High school	19	29.2	14	36.8	5	18.5
University education	11	16.9	8	21.1	3	11.1
No formal education	3	4.6	1	2.6	2	7.4
Missing	15	23.1	3	7.9	12	44.4
Place of residence						
RJ[a] municipality	35	53.8	19	50	16	59.3
Metropolitan area of RJ[a]	54	83.1	17	44.7	2	7.4
Others States of Brazil	1	1.5	–	1	1	3.7
Missing	10	15.4	2	5.3	8	29.6
CD4 count (cell/mm^3)						
> 500			14	36.8		
350–500			2	5.3		
200–349			6	15.8		
< 200			16	42.1		
ART[b]						
Yes			24	63.2		
No			14	36.8		

common symptom.

Additionally, the association between presence or absence of co-infections and the HIV status was also analyzed. Most of the patients (48 of 65; 73.8%) positive for *G. lamblia* infection did not present any co-infection with parasite (monoparasitism), whereas 17 (26.2%) had two or more intestinal parasites simultaneously (polyparasitism). Among the multiple infected, 15 patients had two parasites and the others had more than two parasites. Regarding parasitic associations, 17.6% (3 of 17) of the patients were co-infected by helminths and 82.4% (14 of 17) by protozoa. The only three patients co-infected with helminths were all HIV negative, contrasting with the remaining co-infected patients. For example, patients co-infected with *Cryptosporidium* sp. or *E. coli* were HIV positive (100% and 66.7%, respectively) and all of them were symptomatic (Tables 2 and S1). In HIV infected patients were not observed association between the CD4 T cell count and the presence or absence of polyparasitism.

Twenty patients (30.8%) also have other infections, besides intestinal parasites and HIV virus (Table 2). The bacteria that cause tuberculosis and syphilis were more often found in HIV infected patients (83.3% and 100%, respectively), although our results were not statistically significant. Opposite results were observed with the parasites that cause leishmaniasis and Chagas disease, being more frequent in patients HIV negative.

Of the patients who had hematology and biochemical tests requested, no association were reported with the studied parameters like complete blood count, lipidogramme and proteinogramme (Table S2). However, among the four patients co-infected with *Leishmania*, three had increased concentration of eosinophil in the blood (p = 0.014) (Table S1).

3.3. Associations of G. lamblia assemblages with HIV infections and symptomatology

Of the 65 samples microscopy-positive for *G. lamblia*, 60 (92.3%) were successfully amplified in the multilocus genotyping using at least one locus (*bg*, *gdh*, *orf*C4, *tpi* and/or *ssu rRNA*) (Faria et al., 2016). Thirty-two (53.3%) samples belonged to assemblage A and 28 (46.7%) to assemblage B (Table S1).

In the HIV infected patients, 15 (44.1%) were genotyped as assemblage A and 19 (55.9%) as assemblage B (Table 3). It is noteworthy that among the 28 samples genotyped as assemblage B, 67.9% (19 samples) were HIV infected patients. Despite the observed results are not statistically significant ($p = 0.102$), we could observe an increased prevalence of assemblage B in HIV infected patients.

Regarding the CD4 T cell counts, we could observe that patients with a higher CD4 T cell count (> 200 cell/mm^3) have an equal distribution among the assemblages, 10 (50%) samples belonged to A and 10 (50%) samples to B (Table 4). However, the same was not detected when the CD4 T cell count was below 200 cell/mm^3. In this case, it was observed a greater number of patients (9; 64.3%) harboring assemblage B.

When we studied the association of the symptoms with assemblages, nostatistical association was observed (Table 5). Nevertheless, we could observe a greater number of samples belonging to assemblage B in symptomatic cases, 11 (57.9%) *versus* 8 (42.1%) assemblage A. Conversely, assemblage A was more frequently observed in asymptomatic cases (24 of 41; 58.5%). The most common symptoms in patients with assemblage B were abdominal pain, asthenia, diarrhea, fever, headache and myalgia (Table 5). Rapid weight loss, vertigo and vomiting were more frequent in assemblage A; whereas cough had similar frequency between the assemblages. No association was reported between assemblages and co-infections (Table S1).

The distribution of *G. lamblia* assemblages among the symptomatic and asymptomatic patients according to their HIV status not showed statistically differences (Table 6).

4. Discussion

Previous studies have documented the prevalence of *G. lamblia* among HIV infected patients (Kiros et al., 2015; Marley et al., 2016; Sanyaolu et al., 2011; Tian et al., 2012). However, to the best of our knowledge, this is the first study to provide information on the *G. lamblia* assemblages and symptoms in patients with and without HIV/AIDS virus and the association with CD4 T cell counts worldwide.

There was a positive association between the HIV infected patients and the symptomatology, and diarrhea was the most frequent clinical sign. It is well documented that HIV infected patients have a weakened immune system due to the depletion of CD4 T cells and this makes them more susceptible to a range of infections. Gastrointestinal infections are very common and diarrhea is the hallmark symptom (Akinbo and Omoregie, 2011; Wiwanitkit, 2001). Several studies reported that HIV infected patients were more likely to have intestinal parasitic infection (Nkenfou et al., 2013; Sanyaolu et al., 2011), particularly patients with a CD4 T count below 200 cell/mm^3 (Mehta et al., 2013). Of the non-opportunistic parasites, *G. lamblia* is one of those most commonly found in HIV infected patients (Boaitey et al., 2012; Kiros et al., 2015; Mehta et al., 2013; Nkenfou et al., 2013).

In relation to parasite infections, the majority of the patients seen at INI were infected only with *G. lamblia* (73.8%) and the rest (26.2%) were mainly co-infected by protozoa, which agrees with other studies conducted in Brazil (Castro et al., 2015; Mariano et al., 2015). The low prevalence of helminths observed in our study is probably due to the chemotherapy used in the parasite control programs. Regular deworming with the drugs albendazole and mebendazole is the current control strategy to reduce the prevalence of helminths in Brazil (MS, 2012). However, these programs are not effective against protozoa infections.

Table 2
Clinical information of *Giardia lamblia* infected patients according to HIV status and parasitic co-infections.

Characteristics	Overall (n = 65)		HIV+ (n = 38; 58.5%)		HIV− (n = 27;41.5%)	
	No.	%	No.	%	No.	%
Symptoms[*]						
Yes	21	32.3	16	42.1	5	18.5
No	44	67.7	22	57.9	22	81.5
Clinical symptoms						
Abdominal pain	7	10.8	4	10.5	3	11.1
Asthenia	1	1.5	1	1.5	–	–
Cough	7	10.8	6	15.8	1	3.7
Diarrhea	15	23.1	12	31.6	3	11.1
Fever	8	12.3	6	15.8	2	7.4
Headache	3	4.6	1	1.5	2	7.4
Myalgia	3	4.6	2	5.3	1	3.7
Rapid weight loss	8	12.3	6	15.8	2	7.4
Vertigo	1	1.5	–	–	1	3.7
Vomiting	5	7.7	2	5.3	3	11.1
Intestinal parasitic infection						
Monoparasitism (*G. lamblia*)	48	73.8	31	81.6	17	63
Polyparasitism						
G. lamblia + *A. lumbricoides*	1	1.5	–	–	1	3.7
G. lamblia + *H. nana*	1	1.5	–	–	1	3.7
G. lamblia + *S. mansoni* + *T. trichiura* + *hookworms*	1	1.5	–	–	1	3.7
G. lamblia + *B. hominis*	3	4.6	2	5.3	1	3.7
G. lamblia + *Cryptosporidium* sp.	2	3.1	2	5.3	–	–
G. lamblia + *E. coli*	3	4.6	2	5.3	1	3.7
G. lamblia + *E. histolytica*	1	1.5	–	–	1	3.7
G. lamblia + *E. nana*	4	6.2	1	2.6	3	11.1
G. lamblia + *B. hominis* + *E. histolytica*	1	1.5	–	–	1	3.7
Total of polyparasitism	17	26.2	7	18.4	10	37
Others diseases						
Chagas disease	2	3.1	–	–	2	7.4
Leishmaniasis	4	6.2	–	–	4	14.8
Mycoses	4	6.2	2	5.3	2	7.4
Syphilis	4	6.2	4	10.5	–	–
Tuberculosis	6	9.2	5	13.2	1	3.7

* Statistically significant association among HIV infected patients with symptoms (p = 0.045).

Table 3
Distribution of the *G. lamblia* assemblages among patients over the years.

Assemblage	2011–2012		2013–2015		Total	
	A	B	A	B	A	B
HIV+ (n = 34)	10	6	5	13	15	19
HIV− (n = 26)	13	2	4	7	17	9
Total (n = 60)	23	8	9	20	32	28
p value	0.220		0.694		0.102	

Table 4
Distribution of *Giardia lamblia* assemblages according to CD4 T cell counts in HIV infected patients.

Assemblage	CD4 T cell count (cell/mm^3)				Total
	> 500	350–500	200–349	< 200	
A	5	2	3	5	15
B	7	–	3	9	19
Total	12	2	6	14	34

Table 5
Association between the *G. lamblia* assemblages and symptomatology.

Characteristics	Assemblage		Total	p-value
	A (%)	B (%)		
Symptoms				
Yes	8 (42.1)	11 (57.9)	19	0.235
No	24 (58.5)	17 (41.5)	41	
Clinical symptoms				
Abdominal pain	2 (33.3)	4 (66.7)	6	n.a.
Asthenia	–	1 (100)	1	n.a.
Cough	3 (50)	3 (50)	6	n.a.
Diarrhea	6 (42.9)	8 (57.1)	14	n.a.
Fever	1 (14.3)	6 (85.7)	7	n.a.
Headache	1 (33.3)	2 (66.7)	3	n.a.
Myalgia	–	3 (100)	3	n.a.
Rapid weight loss	4 (57.1)	3 (42.9)	7	n.a.
Vertigo	1 (100)	–	1	n.a.
Vomiting	3 (75)	1 (25)	4	n.a.

n.a.; not applicable.

Co-infections with helminths were only detected in non-HIV-infected patients. Conversely, patients co-infected with *Cryptosporidium* sp. were only observed in symptomatic HIV infected patients, a finding consistent with previous studies that also detected a high prevalence of this opportunistic intestinal parasite in symptomatic HIV patients (Alemu et al., 2011; Sanyaolu et al., 2011). Similarly, we found that *E. coli* a non-pathogenic parasite that is usually seen more frequently in HIV infected patients (Sanyaolu et al., 2011), had a prevalence of 66% in HIV infected patients.

With regard to other co-infections, tuberculosis and syphilis were more frequent in HIV infected patients (83.3% and 100%, respectively). HIV infection is a risk factor for tuberculosis infection and to its progression to an active disease (Getahun et al., 2010; Pawlowski et al., 2012) and syphilis facilitates the transmission and acquisition of HIV infection (Zetola and Klausner, 2007). Leishmaniasis and Chagas

Table 6
Association between *Giardia lamblia* assemblages and symptoms according to HIV status.

Assemblage	HIV +		HIV −		Total	
	A	B	A	B	A	B
Symptomatic	5	9	3	2	8	11
Asymptomatic	10	10	14	7	24	17
Total	15	19	17	9	32	28

disease, however, were only detected in non-HIV-infected patients. While HIV, syphilis and tuberculosis infections are homogeneously distributed in the population of Rio de Janeiro and have a high prevalence (MS, 2014, 2015a, 2015b), leishmaniasis and Chagas disease are not. The reported cases are usually autochthonous, imported from endemic areas of Brazil, attributed to blood transfusion, or travel history, and rarely vertical transmission via the placenta (mother to child), or accidentally in laboratories (Kawa and Sabroza, 2002; Lyra et al., 2015; Sangenis et al., 2015).

No association was found between the socio-demographic variables such as age, gender, place of residence and level of education with the HIV status. Most of the hematology and biochemical parameters between HIV-infected *versus* non-HIV-infected patients were not statistically significant. However, association between *Leishmania* infection and the increased level of eosinophils was detected. This result was unexpected since *Leishmania* infection is associated with Th1 response and macrophage activation (Azeredo-Coutinho et al., 2016; Oliveira et al., 2014).

There was an increased prevalence of assemblage B in HIV infected patients, even though there was no significant statistical association. Analyzing the assemblage's prevalence over the years, we continue to confirm these results. In 2011 and 2012 there was a predominance of assemblage A, with most patients being non-HIV-infected. In the next three years (2013–2015), there was a switch in genetic profile, with an increasing number of patients having assemblage B and most of them being HIV positive. Taking into consideration the CD4 T cell count, a larger number of HIV positive patients with less than 200 cell/mm^3 and *G. lamblia* assemblage B co-infection has been found.

The genetic characterization of *G. lamblia* in HIV infected patients was previously reported in Malaysia, but the relationship between the clinical manifestation and assemblages could not be done because of the small number of positive samples (Lim et al., 2011). One year later, a similar study was conducted in Nigeria and again the small number of positive samples prevented the association being made between the genetic characterization of the parasite and HIV-infected patients (Maikai et al., 2012). Few studies have been conducted on this issue up to now, and, even with the small number of samples analyzed, we can assume that assemblage B is more frequently detected in HIV-infected patients. Corroborating our results, recently, a cross-sectional study of *G. lamblia* infection positively correlated assemblage B with HIV infection (Matey et al., 2016).

Furthermore, we observed a greater number of samples belonging to assemblage B with symptoms (57.9%), particularly abdominal pain, asthenia, diarrhea, fever, headache and myalgia. A correlation between infection with assemblage B and the presence of symptoms has been reported before (Al-Mohammed, 2011; Puebla et al., 2014). Recently, a study conducted in England showed that people infected with assemblage B had more symptoms and greater frequency of vomiting, abdominal pain, swollen stomach, and loss of appetite than people infected with assemblage A (Minetti et al., 2015). In contrast, other studies correlated assemblage A with symptoms (Breathnach et al., 2010; Pestechian et al., 2014; Sahagún et al., 2008).

Some authors believe that the genomic differences between assemblages A and B are sufficient to classify them as separate species, and this could explain some of the phenotypic differences (Adam et al., 2013; Franzén et al., 2009). It has been suggested that different symptom spectra were apparently associated with different assemblages in different populations (Robertson et al., 2010). As aforesaid, several studies were carried out in different countries with different research groups, reporting correlations between assemblages and symptoms. But despite the effort, there is still a lack of concordance on this issue and many questions remain to be answered.

Overall, our results suggest that assemblage B has a good chance of being found in HIV-infected patients and probably the lower CD4 T count is advantageous for assemblage B replication. Moreover, patients harboring assemblage B were more likely to have symptomatic infections than patients with *G. lamblia* assemblage A isolates. It would be particularly interesting proceed the studies about relationship between clinical symptoms, CD4 T cell counts and assemblages of *G. lamblia* in HIV-infected patients. As previously mentioned, HIV infection increases the risk of having intestinal parasitic infections, including *G. lamblia*. The detection and treatment of infections are important measures to improve the quality of life of HIV-infected patients.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actatropica.2017.04.026.

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