Letter to the Editor

Parasitic infections in a Mexican HIV/AIDS cohort

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Opportunistic infections usually aggravate the clinical condition of HIV-infected patients; of these, parasites play an important role as most common causes of morbidity and mortality, especially in those who are severely immunosuppressed [1]. On the other hand, *Blastocystis* is a ubiquitous emerging parasite with unresolved controversy over its pathogenicity; besides, it remains one of the most frequent human intestinal parasites with zoonotic potential. This microorganism has been divided into numerous subtypes (ST), nine of which have been identified in immunocompromised and non immunocompromised human carriers with or without intestinal disorders [2,3].

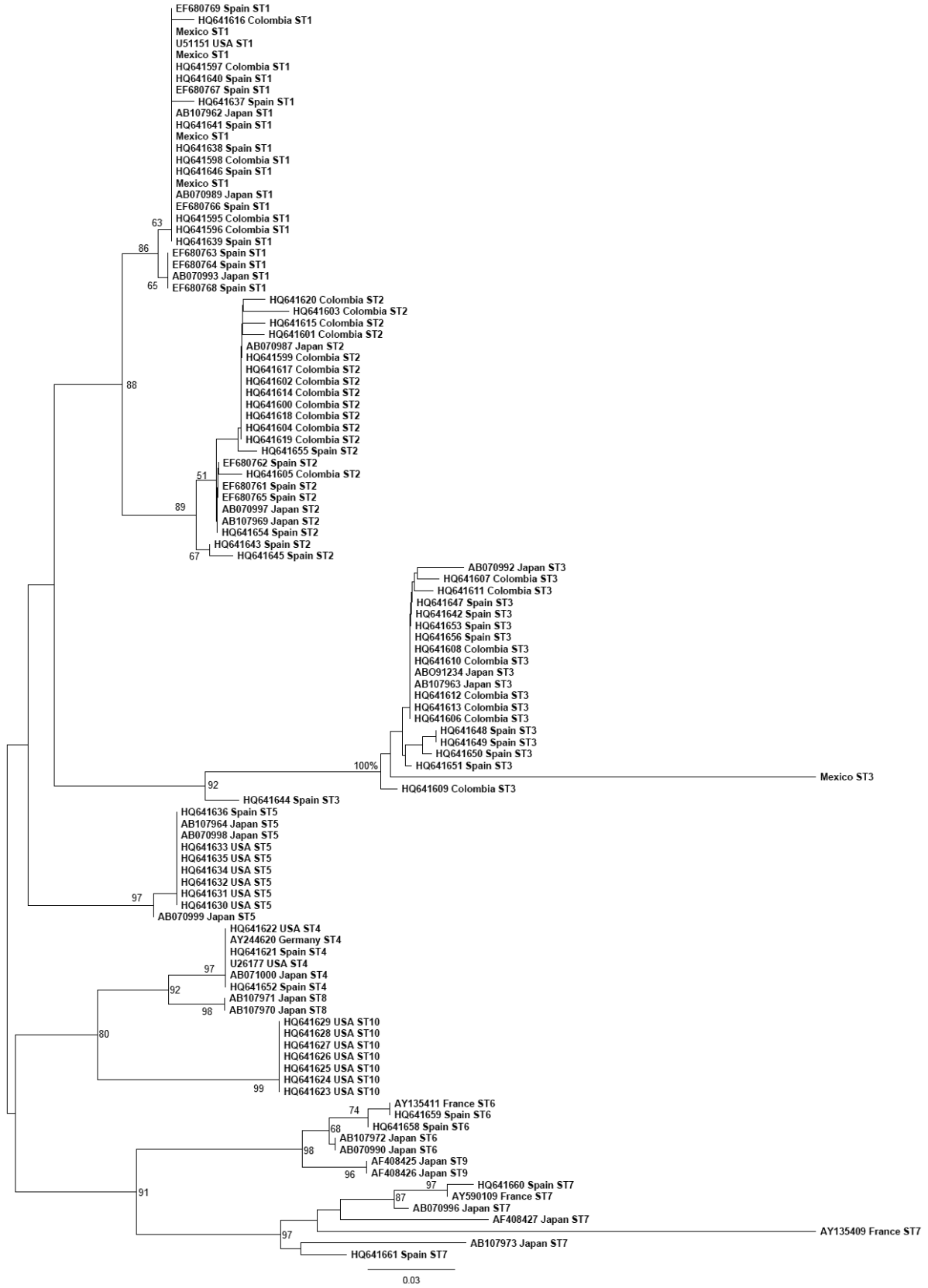
Twenty-seven asymptomatic adult patients confirmed with HIV/AIDS one or two years ago, who have CD4+ T-lymphocyte count >200 cells/mm^3, under highly active antiretroviral therapy (HAART), and are being attended in the HIV/AIDS Clinic of the Hospital General "Dr. Manuel Gea Gonzalez" for their periodic check-up, were recruited for search of opportunistic intestinal parasites and *Blastocystis* ST infection; a descriptive cohort study over a 9-month period (June 2011 to March 2012) was used. The Ethics and Research Committee of our hospital approved the study and informed consent was obtained from each patient before recruitment. For baseline, three faecal specimens were requested to each participant, and four cross-sectional analyses with serial stool samples at one, three, six and nine months were performed. Coproparasitoscopic analysis was performed using Faust´s technique and microscopic observation for search of protozoa cysts and helminth ova was done. Non-concentrated smears were air-dried; methanol fixed and stained using Kinyoun acid-fast to detect *Cryptosporidium spp.*, *Cystoisospora belli* and *Cyclospora cayetanensis* according to Kurniawan et al [1]. Aliquots were processed by PCR for *Blastocystis* diagnosis and identification of ST. For this, stool DNA was extracted and PCR performed according to Jimenez-Gonzalez et al. [4] and Santin et al. [5]; some amplicons were purified and submitted to sequence with a commercial supplier. All sequences were subjected to BLAST search in the GenBank database; multiple alignments were executed and a phylogenetic inference by the neighbor-joining method was performed. Finally, to investigate the association between some variables, a χ^2 test was used. *P*-value <0.05 was considered significant (Epi-Info version 6). Along the study patients who were positive to pathogen parasites were treated conventionally with trimethoprim-sulfamethoxazole for intestinal coccidia and nitazoxanide for protozoa*;* 15 days after treatment serial samples were analysed to corroborate the efficiency to therapy; asymptomatic patients to *Blastocystis* did not receive therapy.

A total of 21 male and 6 female with a mean age and standard deviation of 39±17 years, were recruited. Table 1 shows the positive coproparasitoscopic and PCR results along the study for all participants; eight patients (30%) were negative at the follow-up measurement, while the rest harboured commensal protozoa or by some parasites. In general, participants exhibited one type of infection, while only two presented co-infections (patients 22 and 25). *Blastocystis* was present in 30%, *Endolimax nana* and

Table 1. Parasitological findings of baseline and four cross-sectional analyses, during a cohort study performed over a 9-month period

Patient	Baseline (T0)		1st month (T1)		3th month (T2)		6th month (T3)		9th month (T4)	
	CPS/KINYOUN	*Blastocystis* ST	CPS/KINYOUN	*Blastocystis* ST	CPS/KINYOUN	*Blastocystis* ST	CPS/KINYOUN	*Blastocystis* ST	CPS/KINYOUN	*Blastocystis* ST
1										
2	*C. cayetanensis*									
3	*C. cayetanensis*				*Giardia lamblia*					
4					*Cystoisospora belli*		*Endolimax nana*			
5										
6			*Entamoeba coli*				*Entamoeba coli*			
7	*Blastocystis spp*	ST3								
8	*Endolimax nana*									
9		ST3		ST3		ST3				
10										
11					*Blastocystis spp*	ST1	*Blastocystis spp*	ST1		ST1
12										
13										
14					*Blastocystis spp*	ST3	*Blastocystis spp*	ST3	*Blastocystis spp*	ST3
15					*Endolimax nana*					
16			*Endolimax nana*							
17	*Entamoeba coli*		*Entamoeba coli*	ST1	*Giardia lamblia Entamoeba coli*	ST1	*Entamoeba coli*	ST1	*Entamoeba coli*	
18										
19										
20	*Blastocystis spp*	ST1								
21										
22					*Giardia lamblia/ Endolimax nana*		*Endolimax nana*		*Endolimax nana*	
23			*Cryptosporidiumspp*		*Cryptosporidium spp*					
24			*Blastocystis spp*	ST3						
25			*Endolimax nana Blastocystis spp*	ST1	*Endolimax nana/ Blastocystis spp*	ST1		ST1		
26	*C. cayetanensis*									
27	*Cryptosporidum spp*									

Figure 1. Phylogenetic tree of *Blastocystis* sequences inferred using the neighbor-joining method. GenBank accession numbers and country of origin are shown. Bootstrap proportions (%) are presented in the node branches and those values of less than 50% are not shown.



intestinal coccidia in 22%; other protozoa were less frequent and no helminths were observed. The only statistical association found was between diarrhoea and presence of intestinal coccidia (P=0.004). Half of *Blastocystis* carriers were infected with ST1 and ST3 respectively. All patients infected with coccidia or with *Giardia lamblia* presented intestinal alterations as diarrhoea, abdominal pain and bloating; only two patients (7 and 25) infected with *Blastocystis* showed irritable bowel syndrome according to the Rome III criteria [6]; symptoms declined when anti-parasitic therapy was applied. Interestingly, during the second cross-sectional analysis, patient 17 was symptomatic and presented co-infection with *G. lamblia, E. coli* and *Blastocysti*s, last only identified by PCR; treatment was supplied and 15 days later no parasites were identified and symptoms disappeared, but in the next cross-sectional analysis, *Blastocystis* of the similar ST (ST1) was present together with *E. coli,* probably due to deficient hygienic habits, as suggested by the persistence of *Entamoeba coli*; the patient was asymptomatic. The phylogenetic tree (Figure 1) revealed the presence of ten different clades that corresponded exactly to ST1 to 10 according to Santin et al. [5] and our sequences were grouped in the ST1 and ST3 clades. Although, in present work, controversy remains over the pathogenic potential of *Blastocystis* ST; according to Stensvold et al. [3], it is possible that differences in clinical outcome of *Blastocystis* infection are related to genetic differences on the subtype- or strain-level.

We can conclude that i) *Blastocystis* ST1 and ST3 were the most frequent parasites identified; ii) *Blastocystis* ST1 is potentially capable of reinfecting the same carrier in at least a three-month period; iii) symptomatic cases should be submitted to PCR in order to increase sensitivity in the detection of *Blastocystis,* especially in immunocompromised patients; intestinal coccidia were the most frequent parasites after *Blastocystis;* iv) other molecular markers for *Blastocystis* ST should be studied to elucidate the complexity of this heterogeneous genus and its role in human disease.

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References

1. Kurniawan A, Karyadi T, Dwintasari SW, Sari IP, Yunihastuti E, Djauzi S, Smith HV (2009) Intestinal parasitic infections in HIV/AIDS patients presenting with diarrhoea in Jakarta, Indonesia. Trans R Soc Trop Med Hyg 103: 892-898.
2. Tan TC, Ong SC, Suresh KG (2009) Genetic variability of *Blastocystis sp*. isolates obtained from cancer and HIV/AIDS patients. Parasitol Res 105: 1283-1286.
3. Stensvold CR, Alfellani M, Clark CG (2012) Levels of genetic diversity vary dramatically between *Blastocystis* subtypes. Infect Genet Evol 12: 263-273.
4. Jimenez-Gonzalez DE, Martinez-Flores WA, Reyes-Gordillo J, Ramirez-Miranda ME, Arroyo-Escalante S, Romero-Valdovinos M, Stark D, Souza-Saldivar V, Martinez-Hernandez F, Flisser A, Olivo-Diaz A, Maravilla P (2012) *Blastocystis* infection is associated with irritable bowel syndrome in a Mexican patient population. Parasitol Res 110*:* 1269-1275.
5. Santín M, Gómez-Muñoz MT, Solano-Aguilar G, Fayer R (2011) Development of a new PCR protocol to detect and subtype *Blastocystis spp*. from humans and animals. Parasitol Res 109: 205-212.
6. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC (2006) Functional bowel disorders. Gastroenterol 130: 1480-1491.

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