GENOTYPING OF *GIARDIA LAMBLIA* AND *CRYPTOSPORIDIUM* SPP FROM PANAMANIAN CHILDREN UNDER FIVE YEARS OLD.

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INTRODUCTION

Giardia lamblia and *Cryptosporidium* spp are enteropathogens parasites that can cause gastrointestinal disease and nutritional deficiency in children. These two pathogenic protozoa are present with significant frequencies in rural and suburban communities in Panama, Central America. In some regions of the country it is possible to find giardiasis prevalence between 10-50%. The situation for cryptosporidiosis is less clear. In previous studies we have detected a prevalence between 2-15%. However, information on their genetic characteristics, distribution, and role in human disease in Panama is limited. The molecular characterization of species and genotypes of *Cryptosporidium* and *Giardia* is also necessary for identifying and assessing zoonotic transmission. The present study represents the first contribution in Panama concerning *G. lamblia* and *Cryptosporidium* spp. genotypes circulating in children form different areas of the country.

OBJECTIVE

- To genotype *Giardia* and *Cryptosporidium* isolates from infected Panamanian children under five year of age.

METHODOLOGY

We analyzed the genetic diversity and geographic distribution of both protozoa from infected children younger than five years. Stool samples were taken from **1,840** diarrheic and non-diarrheic children from eight different districts in Panama: Panama, La Chorrera, Cañazas, Santa Fe, Changuinola, Panama Este, Ipeti-Choco and San Felix (Figure 1).

Fig 1: Different Regions of Panama studied for *G. lamblia* and *Cryptosporidium* spp. in children under five years old.

Cysts of *G. lamblia* were microscopically detected using the formalin-acetate concentration procedure. For oocysts detection a Kinyoun staining procedure was used. DNA was extracted only from positive samples. (Fig.2) *G. lamblia* genotyping was performed using a PCR-RFLP analysis based on the polymorphisms of the *tpi* gene. For *Cryptosporidium* spp, the SSU rRNA gene was used as molecular marker. *Cryptosporidium* identification was confirmed by direct sequence analysis. Further genetic diversity within *C. parvum* and *C. hominis* samples were assessed by sequence analysis of the GP60 gene.

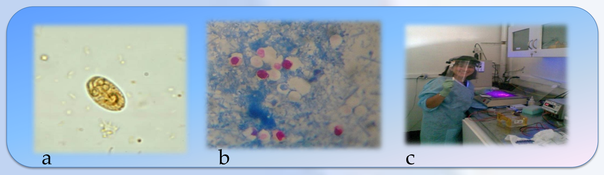

Fig.2: a. Cysts of *G. lamblia* observed in stool samples.
b. Oocysts *of Cryptosporidium* sp.
c .Purification of *Cryptosporidium* samples for genotype analysis.

RESULTS

Of the evaluated samples, 341 (18.5%) presented *G. lamblia* cysts and 93 (5.1%) presented *Cryptosporidium* spp oocysts. DNA was extracted from all of these samples. Molecular diagnosis and characterization was only possible in 132 *Giardia* and 24 *Cryptosporidium* positive samples. Genetic analysis of the tpi gene revealed that of the 132 genotyped *Giardia* samples 25 (19%) belonged to assemblage A, 99 (75.0%) belonged to assemblage B and the remaining 8 (6.1%) were mixed infections (Figures 2 and 3). A preliminary subtyping analysis of assemblage A samples demonstrated that the AII type was nearly nine times more frequent than AI type (Figure 4). Genotyping of 24 *Cryptosporidium* isolates from Panamanian children identified four *Cryptosporidium* species: 62.5% (15/24) were *C. hominis*, 21.0% (5/24) *C. parvum*, 12.5% (3/24) *C. meleagridis* and 4.2% (1/24) *C. canis*. (Table 1, Figure 5). The GP60 gen was sequenced from 15 samples. For *C. hominis* (n =12), 9 belong to the Ie allele, 2 for Id, and 1 for Ib. For *C. parvum* (n =3), two samples were IIc and one IIa .

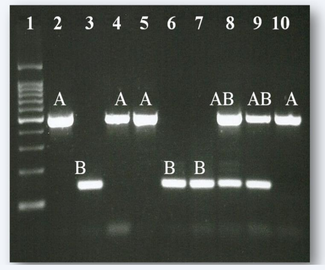

Figure 1. PCR-Multiplex analysis using the tpi gene of *G. lamblia.* Lane 1,100-bp DNA ladder; lane 2, 4, 5 and 10 Assemblage A (476 bp); lanes 3, 6 and 7 Assemblage B (140 bp); lanes 8 and 9 are mixed infections.

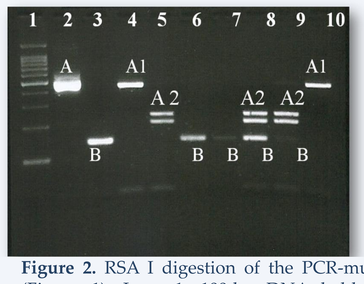

Figure 2. RSA I digestion of the PCR-multiplex products (Figure 1). Lane 1, 100-bp DNA ladder; lanes 4 - 10 correspond to subgenotype A1 (437 bp); lanes 5, 8 and 9 to subgenotype A2. The product of 140 bp, corresponding to Assemblage B was not digested.

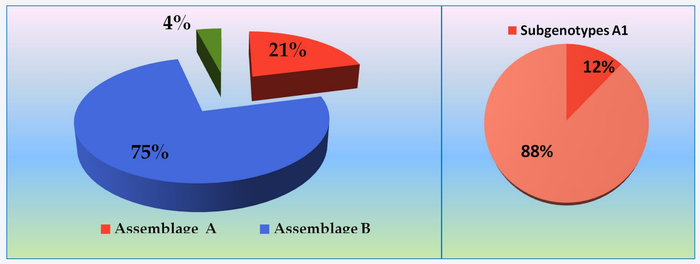

Figure 3. Identification of *Giardia* Assemblages (A and B) and sub-genotypes (A1 and A2) in infected Panamanian children less than five years of age from different regions of the country.

Table I. *Cryptosporidium* genotypes and subtypes from different regions of Panama

SPECIES	REGION	PANAMA	CHORRERA	CHANGUINOLA	SANTA FE	CAÑAZAS	SAN FELIX	CHEPO
Total	N=24	N=3	N=6	N=5	N=2	N=2	N=5	N=1
C. hominis	n=16	1	5*	5	1*	1	3*	
subtype family	Ie	1	3	4			1	
(12/16)	Id			1		1		
	Ib		1					
C. parvum	n=4							1*
subtype family	IIa		1					
(3/4)	IIc	2						
C. meleagridis	n=3				1		2	
C.canis	n=1					1		

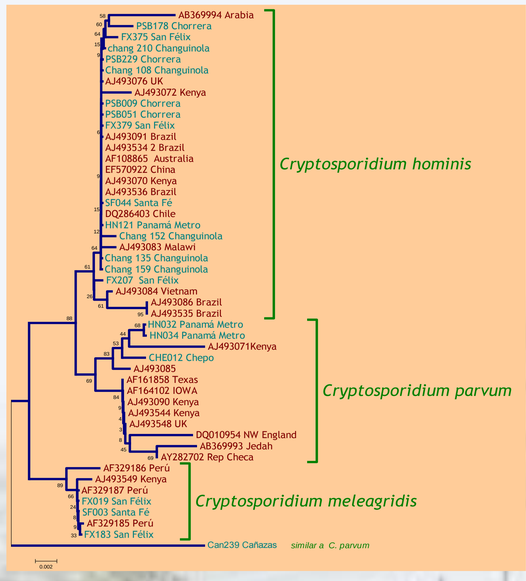

Figure 5. Phylogenetic relationships of the examined isolates with other *Cryptosporidium* genotypes as inferred by Neighbor-joining analysis, based on the nucleotide sequence of SSU rRNA. The name of the isolates accession numbers in GenBAnk are shown in red.

CONCLUSIONS

- *G. lamblia and Cryptosporidium* spp. are important protozoan infections in children from different regions of Panama. *G. lamblia* assemblage B (75%) is the main genotype and *Cryptosporidium hominis* (62.5%) the predominant species circulating in different regions of Panama. In addition, other potential zoonotic *Cryptosporidium as C. meleagridis* (12.5%) *and C. canis* (4.2%)were detected. *C. hominis* Ie and *C. parvum* IIc were the predominant subtypes circulating in Panama.
- Further studies of human and animal isolates are required to evaluate the molecular epidemiology of *G. lamblia* and *Cryptosporidium* spp. in Panama. This information is important for the development of systematic prevention and control measures for these enteroparasitic infections.