RESEARCH NOTE

Giardia lamblia: Isolation, Axenization and Characterization of a Strain from an Asymptomatic Patient from Belo Horizonte, MG, Brazil

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Giardia lamblia is a protozoan parasite which inhabits the small intestine of man and other animals. It causes a spectrum of symptoms that varies from asymptomatic to acute or chronic diarrhea with malabsorption (MJG Farthing 1989 *Q J Med 70*: 191-204, R Cedillo-Rivera et al. 1991 *Arch Invest Med (Mex) 22*: 79-85). The infection in children is sufficient to interfere with growth and development. It has a worldwide distribution with a prevalence varying between 2 and 5% in industrialized countries and up to 20-30% in the developing ones (Farthing *loc. cit.*). *G. lamblia* is the intestinal parasite most frequently identified in public health laboratories in the United States and in the United Kingdom (DE Einfeld & HH Stibbs 1984 *Infect Immun 46*: 377-383, EA Meyer & E Jarrol 1980 *Am J Hyg 111*: 1-12). *G. lamblia* from human and other animals, isolated from diverse geografic areas, have been established in culture and maintained axenically (BLP Ungar & TE Nash 1987 *Am J Trop Med Hyg 37*: 283-289). Differences among the various isolates have been reported by several authors, defined by surface antigen analysis (Ungar & Nash *loc. cit.*), growth curves (PD Smith et al. 1982 *Infect Immun 36*: 714-719, Cedillo-Rivera et al. *loc. cit.*), isoenzymes profiles (SH Korman et al. 1986 *Z Parasitenkd 72*: 173-180, BP Meloni et al. 1988 *Am J Trop Med Hyg 38*: 65-73, UK Baveja 1986 *Aust J Exp Biol Med Sci 64*: 119-126) and DNA banding pattern (Ungar & Nash *loc. cit.*). The biological importance of these differences remains unkown, but it could alter the host-parasite relationship and might also modify the immune response or resistance of the host (TE Nash 1989 *Exp Parasit 68*: 238-241, TE Nash & DB Keister 1985 *J Infect Dis 152*: 1166-1171).

Here we report the isolation, axenization and characterization of a *G. lamblia* strain from Belo Horizonte, MG, Brazil. Growth curves, polyacrylamide gel electrophoresis of protein extract and isoenzymes profiles were performed and compared with the axenic Portland strain (ATCC30888).

Cysts of *G. lamblia* were obtained from the feces of a 4-year old asymptomatic child, living in Belo Horizonte. The cysts were isolated and concentrated by centrifugation on a sucrose gradient, according to I.C. Roberts-Thomson (1976 *Gastroenterol 71*: 57-61). Approximately 1 x 10^6 cysts were treated with floxacin (2.0 mg/ml) and nistatine (1.000 U/ml) dissolved in 5.0 ml of a 2% HCl solution for seven days, at 4°C. The cysts were subsequently washed five times by ressuspending them in distilled water after centrifugation at 1.000g for 5 min. Washed cysts were inoculated into 15 ml of TYI-S-33 culture medium supplemend with bovine bile (0.06%) and 250µg/ml streptomycin and 200 U/ml penicilin G, and incubated at 37°C. Cultures, examined three days later, had abundant trophozoites. Subculturing in the same medium was done. After the third passage, antibiotics were not included in culture media. Culture samples, inoculated into thioglycolate and agar-blood were passaged five times to confirm axenicity. The isolate was then considered to be axenic and designated BHRF92. The growth curve of BHRF92 strain was similar to that obtained for the Portland strain. Protein extracts from both strains were submitted to polyacrylamide gel electrophoresis and presented qualitatively and quantitatively distinct profiles (Fig. 1). Comparison of the electrophoretic profiles of trophozoites suggest that there is a considerable antigen heterogeneity between the BHRF92 strain and the Portland. The isoen-zymatic characterization was performed using malate dehydrogenase (MDH) (EC1.1.1.37), 6 phosphogluconate dehydrogenase (6PGDH) (EC1.1.1.44), glucose-6-phosphate dehydroge-nase (G6PDH) (EC1.1.1.49), glucose phosphate isomerase (GPI) (EC5.3.1.9.), phosphoglucomu-tase (PGM) (EC2.7.5.1.), alanine aminotransfe-rase (ALAT) (EC2.6.1.2.) and aspartate amino-transferase (ASAT) (EC2.6.1.1.).

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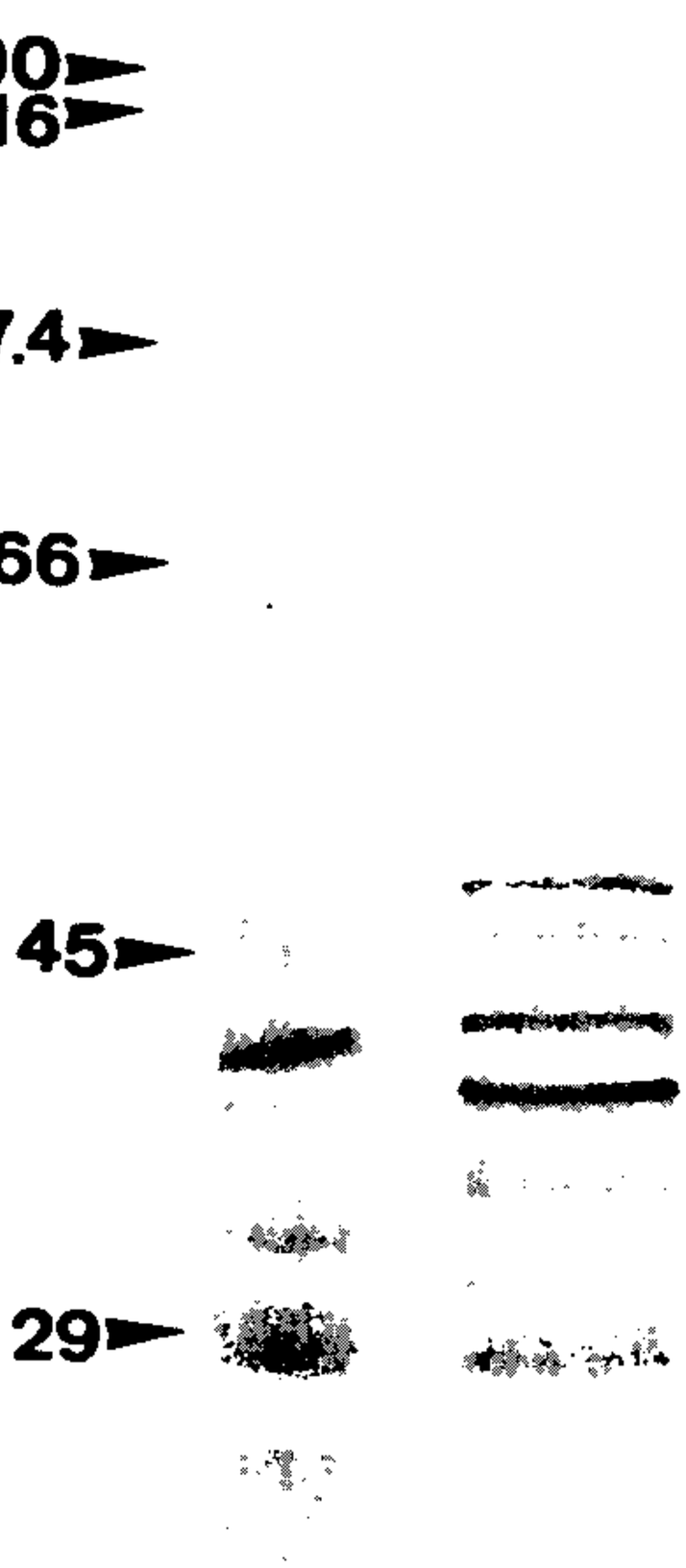

Fig. 1: photograph of the electrophoretic profiles obtained with soluble extracts of *Giardia lamblia* cultured trophozoites (1) BHRF92 strain (Minas Gerais, Brazil) and (2) Portland strain (ATCC30888).

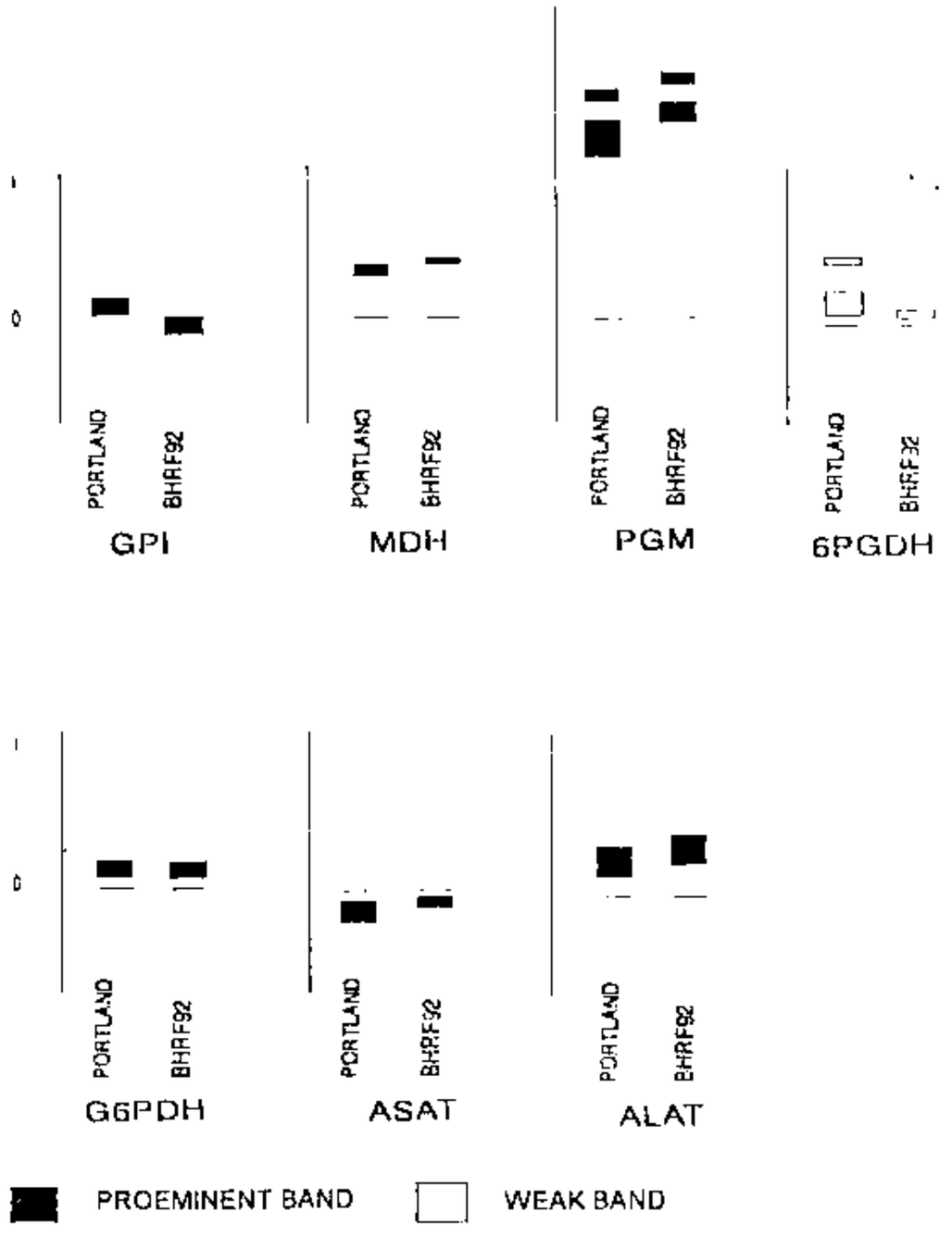

Fig. 2: diagramatic representation of isoenzyme profile of *Giardia lamblia* BHRF92 strain (Minas Gerais, Brazil) and Portland strain (ATCC 30888) using seven enzyme systems. For abbreviations, see text.

The isoenzyme analysis revealed differences in six enzyme patterns (GPI, MDH, PGM, 6PGDH, ASAT and ALAT) and homogeneity in one enzyme (G6PDH). The GPI and 6PGDH presented more remarkable differences. (Fig. 2). The two strains showed distinct zymodemes as has been demonstrated for others *G. lamblia* strains from various geographical location (MA Betram et al. 1983 *J Parasit 69:* 793-801, Meloni *loc. cit.*).

This is the first report on the isolation, axenization and characterization of *G. lamblia* from Minas Gerais. The differences in *G. lamblia* strains can be correlated with variable clinical manifestations, host responses and treatment efficacy of human giardiasis (Korman *loc. cit.*). Also, the differences could be important for clinical diagnostic and therapeutic. These preliminary data present information on a strain of *G. lamblia* that could be taken as a reference for further comparisons.