Parasitol Res (2008) 103:459–465
DOI 10.1007/s00436-008-1007-3

ORIGINAL PAPER

Prevalence of enteroparasites and genotyping of *Giardia lamblia* in Peruvian children

G. Peréz Cordón · O. Cordova Paz Soldan · F. Vargas Vásquez · J. R. Velasco Soto · Ll. Sempere Bordes · M. Sánchez Moreno · M. J. Rosales

Received: 10 March 2008 / Accepted: 16 April 2008 / Published online: 11 May 2008

Abstract Enteroparasites in children from three marginal urban districts of Trujillo (Peru) were studied to treat these children and to design a prevention and control programme. A total of 845 children were examined. The general prevalence of enteroparasites was of 66.3%, and 45.6% were multiparasitized. The pathogenic enteroparasite prevalence were 23.8% (*Giardia lamblia*), 4.6% (*Iodamoeba buschlii*), 2.6% (*Cyclospora cayetanensis*), 2.2% (*Hymenolepis nana*), and 2% (*Cryptosporidium* spp.). *G. lamblia* was the most frequent parasite both in diarrheic children (28.1%) as well as in nondiarrheic ones (19.5%). The *G. lamblia* genotypes were molecularly characterized by sequence analysis of the glutamate dehydrogenase (gdh) gene using PCR and RFLP. Sequence analysis revealed both Assemblage A (AI and AII) and Assemblage B (BIV), with the predominance of Assemblage AI. All the samples with Assemblage A were diarrheic but not those with Assemblage B. This is the first study of molecular characterization of *G. lamblia* in Peruvian children and confirms the importance of asymptomatic patients in the transmission of the giardiosis, especially in places with poor hygiene and sanitation.

G. Peréz Cordón · J. R. Velasco Soto · Ll. Sempere Bordes · M. Sánchez Moreno · M. J. Rosales (✉)
Instituto de Biotecnología, Departamento de Parasitología, Facultad de Ciencias, Universidad de Granada, Severo Ochoa s/n, 18071 Granada, Spain
e-mail: mjrosale@ugr.es

O. Cordova Paz Soldan · F. Vargas Vásquez
Instituto de Investigación en Microbiología y Parasitología Tropical, Facultad de Ciencias Biológicas, Universidad Nacional de Trujillo, Trujillo, Perú

Introduction

In Peru, infections from intestinal parasites are frequent in children, raising the need for prevention programs, control, and treatment of intestinal parasites. Data from 1992 indicated that the prevalence of enteroparasitosis in Latin America ranged from 20% to 30% for the general population, and 60% to 80% or populations with high endemic rates (Savioli et al. 1992).

Currently the Pan American Health Organization (PAHO), through different regional programs, is fighting against the so-called parasitic and unattended diseases. These diseases include intestinal parasitoses, primarily geohelminthioses caused by *Ascaris lumbricoides, Trichuris trichura, Ancylostoma duodenale* and *Necator americanus*. The last study of the PAHO on infection by geohelminths in the Latin American population, dating to 1998, estimates that 30% of the population is affected, while the data on the poor neighborhoods reach a frequency of 50% and even 95% in some indigenous tribes (PAHO 1998).

Children constitute the sector most affected by intestinal parasites. Concerning the morbidity that this causes, the World Health Assembly, in Resolution 54.19 (2001) indicated that at least 75% of school-age children living in high-risk areas for geohelminths and *Schistosoma* will need access to regular chemotherapy for the year 2010. Therefore, the WHO has for years been providing incentives, such as the Projects of Healthy Schools (WHO 1996).

Several studies show the importance of enteroparasites in Peru. The first works published appeared in Gonzales-Mugaburu (1956) and Torres-Portugal and Campos (1960), in which the incidence of the intestinal parasites was in the preschool population of the city of Arequipa. Of the parasitoses, 79.9% were caused by protozoa and the rest by helminths, the incidence of intestinal *Giardia* being 18.9%,

Hymenolepis nana 11.4%, and *Entamoeba histolytica* 3.4%. From these studies to the present, there do not appear to be many changes. Thus, the most recent study on Puno (Maco et al. 2002) found an intestinal parasitosis of 91.2%.

In the city of Trujillo, the object of the present study, Vargas and Castillo (1987) found that the protozoa and helminths exerting the greatest influence in the urban, suburban, and rural zones, respectively, were *Entamoeba coli* and *H. nana*. Vargas and Castillo (1987) established a prevalence of 30% for *Giardia* in children under 10 years of age with acute diarrhea. Recently, Cordova et al. (2006) found the prevalence of intestinal parasitoses to be some 68%. The most frequent pathogenic enteroparasites were *Giardia* (26.4%), *Cyclospora cayetanensis* (13%), *H. nana* (2%), *Hymenolepis diminuta* (1.6%), and *Cryptosporidium* spp. (1%).

Giardia lamblia is thus one of the most frequent enteroparasites in Peruvian children, as shown by these studies in Trujillo and those performed by Berkman et al. (2002) in Lima. The incidence in Lima was 0 to 4.8 per year during infancy, with 86% of the children having a least one *G. lamblia* episode during the first 2 years of life.

Isolates of *G. lamblia* can be grouped into a number of recognized genotypic clusters or assemblages, based on analysis of conserved genetic loci (Thompson et al. 2000). The assemblages include A and B, which are potentially zoonotic and C, D, E, F, and G, which appear to be host restricted (Monis and Thompson 2003). Only genetic assemblages A and B isolates have been recovered from humans and a broad range of hosts.

The glutamate dehydrogenase (gdh) gene is proving useful for the genotyping of *Giardia* isolates from mammals. By sequence analysis of this locus, isolates can be grouped into their genetic assemblage and also into subgroups AI, AII, BIII, and BIV (Monis et al. 1996).

The Agencia Española de Cooperación Internacional (AECI) and the Agencia Andaluza de Cooperación Internacional (AACI) during the years 2004 and 2005 have financed a project of prevention, control, and treatment of intestinal parasites in Trujillo, to lower the prevalence of parasitic infections among children.

This work shows the prevalence of intestinal parasites in undernourished children from three districts of Trujillo (Peru) during 2005, as well as the molecular epidemiology of *G. lamblia*.

Materials and methods

Study site and sampling

Trujillo is one of the 12 provinces of the Department of La Libertad, Peru. It is bordered on the north by the province of Ascope, to the east by Otuzco, to the south by Virú, and to the west by the Pacific Ocean. According to the last census of population and housing (2005), the city of Trujillo has some 800,000 inhabitants, of which 50% live in the district of La Libertad.

The study was performed in the four schools situated in three marginal urban districts on the periphery of the city of Trujillo: La Esperanza, El Povenir, and Buenos Aires. The three districts have similar living conditions, in that not all the dwellings have running water, and thus the inhabitants get water from public fountains or water trucks, and most of the houses lack toilets.

From January to December 2005, stool samples were collected throughout the four seasons of the year from children between 1 month and 9 years old to study intestinal parasites in the schools of each district.

Analysis

The samples for the examination of the intestinal parasites were processed as in Cordova et al. (2006). Samples were transported to the laboratory and preserved in potassium dichromate at 2.5% and kept at 4°C until macroscopically and microscopically examined using a binocular microscope (using lugol in some cases).

All the samples were stained with Ziehl-Neelsen, Giemsa, and Heidenheim and analyzed by the Kato-Katz method for nematode ova (WHO 1991).

Fecal specimens were initially screened for the presence of *Giardia* using zinc sulfate flotation. The Teleman concentration was used when other parasites were detected.

Data were compared between diarrheic and nondiarrheic children using χ^2 test. Prevalence of intestinal parasites was studied having a P value<0.02.

Molecular characterization of *Giardia duodenalis* genotypes

DNA extraction

All the positive fecal samples for *Giardia duodenalis* were processed for DNA extraction.

Cysts were disrupted using five freeze-thaw cycles (dry-ice ethanol bath at 65°C). Afterwards, 200 µl of fecal sample in water was suspended in 200 µl of lysis buffer supplied in the QIAamp DNA mini kit (QIAGEN, USA). Genomic DNA was isolated by a QIAamp DNA stool mini-kit protocol (QIAGEN) directly from the fecal sample (200 µl/sample). DNA samples were stored at −20°C until further use.

PCR-RFLP of region of the glutamate dehydrogenase gene

Giardia duodenalis genotypes were determined by nested polymerase chain reaction (PCR) and restriction fragment

length polymorphism (RFLP) analysis as previously described (Read et al. 2004) using *Nla*IV and *Rsa*I. For the nested PCR step, a fragment of approximately 432 base pairs of the gdh was amplified by using external forward primer GDHeF (TCA ACG TYA AYC GYG GYT TCC GT), internal forward primer GDHiF (CAG TAC ACC TCY GCT CTC GG), and reverse primer GDHiR (GTT RTC CTT GCA CAT CTC C; Invitrogen, Spain).

PCR reaction mixtures consisted of 12.5 pmol of each primer, 200 μm of each dNTP, 1.5 mM MgCl2, 0.1 U of Taq polymerase (BioRad, Spain) and PCR buffer. The reaction was carried out in 25 μl volumes. GDHeF and GDHiR were used in the primary PCR reaction with 1 μl of genomic DNA. One microtiter of PCR product from the primary reaction was added to the secondary PCR containing primers GDHiF and GDHiR. Each PCR was performed in a thermocycler (MyCycler, BioRad) with the following amplification conditions: 1 cycle of 94°C for 2 min, 56°C for 1 min and 72°C for 2 min, followed by 55 cycles of 94°C for 30 s, 56°C for 20 s, and 72°C for 45 s. A final extension of 72°C for 7 min and a 4°C hold. Restriction digests were carried out directly on PCR products in 20 μl reactions. Ten microliters of PCR product were added to 1× reaction buffer and 2 U NlaIV (New England Biolabs, Spain) or 2 U RsaI (New England Biolabs, Spain), and digestion took place at 37°C for 3°h.

The PCR products and a ladder of 1,000 bp (Sigma, USA) were electrophoresed in a 2.0% agarose gel and visualized by ethidium bromide staining.

Reactions were visualized on ethidium bromide stained 2.0% agarose gels.

As a positive control, a sample of assemblage AI of *G. lamblia* (ATCC 30888) was used.

Results

Epidemiological study

A total of 845 fecal samples were collected and analyzed: 251 from El Porvenir, 425 from La Esperanza, and 169 from Buenos Aires. The median age of the children was 6 years (interquartile range 4–8). One or more intestinal parasitic infections were identified in 560 (66.3%) of the children, 45.6% of them having multiple parasitic infection by two, three, or four parasites. The protozoa detected (Table 1) were *G. lamblia*, *C. cayetanensis*, *Cryptosporidium* spp., *Entamoeba coli*, *Endolimax nana*, *Iodamoeba buschlii*, *Blastocystis hominis*, and *Chilomastix mesnilii*. The helminths were *H. nana*, *Enterobius vermicularis, A. lumbricoides, T. trichura*, and *Dyphyllobotrium* sp. In the three districts studied, the parasite prevalence proved similar: *G. lamblia* (23.8%), *Iodamoeba buschilii* (4.6%), *C. cayetanensis* (2.6%), *H. nana* (2.2%), and *Cryptosporidium* spp. (2%).

Table 1 shows the general prevalence of each parasite and the prevalence for both populations, children with and without diarrhea (defined as a change in the normal pattern of bowel movements, with at least three loose stools daily).

All the parasites except for *Dyphylobotrium* spp. were found in the fecal samples both from diarrheic children as well as from nondiarrheic children. Some, such as *E. vermicularis, E. nana*, or *Iodamoeba buschilii* showed greater prevalence in nondiarrheic children than in diarrheic children. The prevalence of *G. lamblia, C. cayetanensis, Cryptosporidium* spp., *A. lumbricoides*, or *H. nana* in nondiarrheic children was also very high although lower than in diarrheic children.

Table 1 Prevalence of intestinal parasites during 2005 in children of Trujillo (Peru)

Parasite	Diarrheic children, *n* (%)	Nondiarrheic, *n* (%)	Prevalence (%)
B. hominis	147 (33.3)	165 (37.5)	35.4
Giardia intestinalis	124 (28.1)	86 (19.5)	23.8
Entamoeba coli	49 (11.1)	81 (18.4)	14.7
E. nana	39 (8.8)	40 (9.1)	8.9
I. buschlii	17 (3.8)	24 (5.5)	4.6
C. mesnilii	18 (4.1)	16 (3.6)	3.9
C. cayetanensis	15 (3.4)	8 (1.8)	2.6
Cryptosporidium spp.	14 (3.2)	4 (0.9)	2
A. lumbricoides	4 (1)	2 (0.5)	0.7
Thichuris trichiura	1 (0.2)	1 (0.2)	0.2
E. vermicularis	2 (0.5)	5 (1.4)	0.8
H. nana	12 (2.7)	7 (1.6)	2.2
Dyphyllobotrium spp.	0	1 (0.2)	0.1

Table 2 The most frequent multiple infections by intestinal parasites in children of Trujillo (Peru) during 2005

	No. of cases
Two parasites	
G. intestinalis + *B. hominis*	53
E. coli + *B. hominis*	29
B. hominis + *E. nana*	14
B. hominis + *C. Mesnilii*	8
E. coli + *E. nana*	5
B. hominis + *I. buschlii*	5
E. coli + *C. cayetanensis*	4
G. intestinales + *E. nana*	3
E. coli + *I. buschlii*	3
E. coli + *Cryptosporidium spp.*	2
G. intestinalis + *C. cayetanensis*	2
G. intestinales + *H. nana*	2
Three parasites	
E. coli + *B. hominis* + *G. intestinalis*	12
G. intestinalis + *B. hominis* + *E. nana*	9
E. coli + *B. hominis* + *E. nana*	7
G. intestinalis + *B. hominis* + *I. buschlii*	7
G. intestinalis + *B. hominis* + *C. mesnilii*	3
B. hominis + *I. buschlii* + *E. coli*	3
E. coli + *B. hominis* + *C. mesnilii*	3
E. coli + *G. intestinalis* + *I. buschlii*	2
E. coli + *B. hominis* + *H. nana*	2
B. hominis + *I. buschlii* + *E. nana*	2
Four parasites	
E. coli + *B. hominis* + *C. mesnilii* + *G. intestinalis*	2
G. intestinalis + *B. hominis* + *I. buschlii* + *E. coli*	2

Multiple parasitic infections were common (Table 2). Two, three, or four intestinal parasites were identified in 45.6% of the children. The most frequent cases of multiple parasitism were *G. lamblia* together with *B. hominis* (53 cases), and three parasites together, *E. coli*, *B. hominis*, and *G. lamblia*. Serious diarrheic processes resulted in the cases of multiple parasitic infections for *G. lamblia* + *C. cayetanensis* and *G. lamblia* + *B.hominis* + *E. nana.*

Molecular characterization of *Giardia*

Of the 845 children analyzed, 210 presented *G. lamblia*, 124 had diarrhea, and 86 did not. All the positive fecal samples for *Giardia lamblia* were processed for DNA extraction, but only in 16 could we appreciate a band of 432 bp. PCR products of these 16 fecal samples were digested with *Nla*IV and *Rsa*I.

Of these samples, nine generated three visible bands of 90, 120, and 150 bp (Fig. 1), indicating assemblages AI of *G. lamblia*, as control in lane 2. Six samples generated two bands of 120 and 290 bp, this being either the assemblage BIV or BIII of *G. lamblia*. Only one fecal sample generated four bands (70, 80, 90, and 120 bp), indicating assemblage AII.

The six samples belonging to the assemblages BIV or BIII were digested with RsaI and all of this generated a band of 430 bp, thus indicating assemblage BIV (Fig. 2).

Discussion

In Peru, parasitoses of the digestive tract have been widely studied because they are a very frequent public-health problem and can affect the growth and development of school children. The present study determines the intestinal parasites affecting children from three districts of Trujillo (Peru) during the year 2005 as well as the most frequent *G. lamblia* genotypes.

The prevalence of intestinal parasites in children in this study, during 2005, was very high (66.3%), similar to those found in the year 2004 in these districts (68%; Cordova et al. 2006). Other studies reflect 91.2% prevalence of enteroparasites in children en Puno, Peru (Maco et al. 2002)

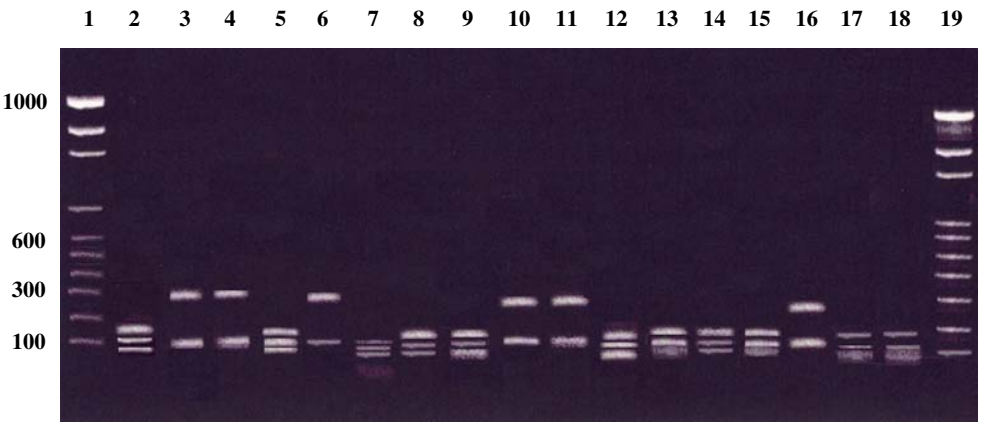

Fig. 1 Ethidium bromide stained 2% high-resolution agarose gel showing DNA amplified at the gdh from each of the genetic Assemblages AI, AII and B digested with Nla IV. *Lanes 1 and 19*, molecular-weight marker (1,000 bp); *lane 2*, positive control of assemblage AI of *G. lamblia* (ATCC 30888); *lanes 5, 8, 9, 12, 13, 14, 15, 17, 18* Assemblage AI of *G. Lamblia*; *lane 7* Assemblage AII; *lanes 3, 4, 6, 10, 11, 16* Assemblage B

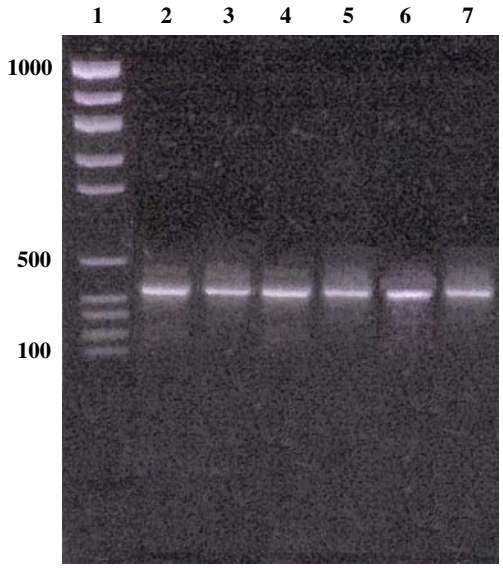

Fig. 2 Ethidium bromide stained 2% high-resolution agarose gel showing DNA amplified at the gdh from the genetic Assemblages B digested with Rsa I. *Lane 1*, molecular weight marker (1,000 bp); *lanes 2, 3, 4, 5, 6, 7* Assemblage BIV

and 54.7% en Santiago de Surco, Lima, Peru (Iannacone et al. 2006).

The results for Trujillo for 2005 may be surprising as the children affected by pathogenic enteroparasites during 2004 had been prescribed proper treatment by the health centers of each district. It is possible that this treatment was not followed correctly or else that after the treatment the children were quickly reinfected. This latter fact was demonstrated some time ago in studies made in Lima, Peru, where the high rate of reinfection for *G. lamblia* after therapy and long duration of parasite excretion alter reinfection all suggest that children in developing countries do not acquire immunity to this parasite (Gilman et al. 1988).

In the three districts studied, the parasite prevalence proved similar (Table 1): *G. lamblia* (23.8%), *Iodamoeba buschilii* (4.6%), *C. cayetanensis* (2.6%), *H. nana* (2.2%), and *Cryptosporidium spp*. (2%). In 2004 the prevalence of this parasites were 26.4% *G. lamblia*, 10.2% *I. buschlii*, 13% *C. cayetanensis*, 2% *H. nana*, 1.6% *H. diminuta*, and 1% *Cryptosporidium* spp. (Cordova et al. 2006). Thus, in all the pathogenic enteroparasites the prevalence declined during 2005 with respect to 2004, after the treatment of the children, but this was especially evident in the case of *I. buschlii* (4.6% vs. 10.2) and *C. cayetanensis* (2.6% vs. 13%). In both cases, intestinal cestodosis was low possibly because the basic foods of these children are vegetables and carbohydrates. Only *Dyphyllobothrium* spp. was found in this study due to the widespread custom of consuming ceviche, prepared with raw fish, but the prevalence was low (0.1%).

The high prevalence of *G. lamblia* (23.8%) was striking, causing diarrhea in 28.1% of the children and failing to cause it in 19.5%. It has been demonstrated that the *Giardia* cysts isolated from the feces of asymptomatic children, and used to infect gerbils, were more infective than those from symptomatic children (Astiazaran et al. 2000). Our results underscore the importance of asymptomatic children as carriers in communities with poor hygiene and deficient control over water or food. In any case, the prevalence of *G. lamblia* has diminished somewhat with respect to previous studies conducted in Trujillo in 1987 (Vargas and Castillo 1987) where infection reached 30%.

The Puno study (Maco et al. 2002) reported 6.6% *H. nana*, 5.5% *Entamoeba histolytica*, 3.3% *G. lamblia*, 2.2% *Taenia* sp., 2.2% *A. lumbricoides*, 1.1% *T. trichura*, and 1.1% *E. vermicularis*. In Santiago de Surco district, Lima, Peru (Iannacone et al. 2006), the most prevalent enteroparasites were *E. vermicularis* (10.4%), *G. lamblia* (4.7%), *Ancylostoma duodenale–Necator americanus* (1.6%), and *A. lumbricoides* (1.6%). In both works, the prevalence was far lower for *G. lamblia* than in Trujillo.

Multiple parasitism was frequent (Table 2), with 45.6% of parasitized children presenting two, three, or four parasites. The most frequent association was *G. lamblia* and *B. hominis* (53 cases) in children with acute diarrhea.

G. lamblia is one of the major diarrhea agents in human and animals distributed worldwide and the infection has a major clinical impact on children that are 5 years old or younger (Adam 2001; Thompson 2000). The prevalence of this infection varies between 2 and 5% in industrialized countries and up to 20–30% in less developed countries, amounting to 200 million symptomatic cases in Asia, Africa, and Latin America with an incidence of 500,000 new cases per year (CDC 1995).

The present study reflects that *G. lamblia* is the most frequent pathogenic enteroparasite in the infantile population studied, with a very high prevalence (23.8%) and causing acute diarrhea in 28.1% of the cases. *G. lamblia* presented high levels of genetic diversity, showing seven genotypes: A, B, C, D, E, F, and G. Only Assemblages A and B have been detected in humans and in a wide range of other mammalians hosts, whereas the remaining assemblages (C–G) are host specific (Volatao et al. 2007). Molecular characterization of cysts of human and animal origin are useful to address the co-circulate isolates between these host, and represents an objective means of evaluating a zoonotic-infection hypothesis.

In the present study, *G. lamblia* genotypes were determined by PCR-RFLP in the region of the glutamate dehydrogenase (gdh) gene, as described by Read et al. (2004), using *Nla*IV and *Rsa*I. This technique was selected on the basis of being able to differentiate subgroups (AI, AII, BIII, and BIV) within the genetic assemblages. By this

method, only 16 samples gave a fragment of 432 bp of the gdh. This could be because to develop this technique, a great quantity of cysts is needed, and this is not always possible in all the fecal samples, although the concentration of the cysts is taken. The most frequent Assemblage was AI in nine of the samples processed, followed by BIV in six samples, and by AII in only one. It was not possible to differentiate clearly between the BIV and BIII assemblages after digestion with RsaI, and therefore all six samples that offered bands of 120 and 290 bp afterwards gave a fragment of 430 bp corresponding to Assemblage BIV.

To date, no study has been made in Peru for the molecular characterization of *G. lamblia* isolates, although Sulaiman et al. (2003), to test the effectiveness of the triosephosphate isomerase gene characterization, used isolates of different origins; those from Lima revealed both human Assemblages A and B, with a clear predominance of Assemblage B. The greatest zoonotic risk is from Assemblage A *Giardia* genotype and to a lesser degree from Assemblage B genotype, which appears to be predominantly human-specific (Thompson 2004). In our study, Assemblage A predominates, and we cannot determine whether the transmission was zoonotic or not, but we can indicate that there is a high risk of anthroponotic transmission, as six samples presented Assemblage B. This transmission in children can be attributed to their habit of defecating on the ground in the absence of toilets in houses and schools, thereby contaminating water and food with human excrement.

Recently, a likely association has been reported between Assemblage A infections and increased odds ratios for diarrhea, whereas higher parasite-DNA loads and a higher overall prevalence were observed for Assemblage B infections, statistically related to asymptomatic *Giardia* infection (Haque et al. 2005). Our results agree with this, as the 11 samples with Assemblage A were diarrheic, and those corresponding to Assemblage B, despite containing high quantities of *G. lamblia* cysts, were nondiarrheic. Therefore, with the results found, we continue to underline the importance of the asymptomatic children in the transmission of *G. lamblia* both directly as well as indirectly.

The present work demonstrates the high prevalence of intestinal parasites in Peruvian children, the easy reinfection of the children with these parasites, and the high prevalence of *G. lamblia*, one of the most pathogenic enteroparasites in children. Based on the results of this study, control and prevention programmes are being set up against the most frequent intestinal parasites.

Acknowledgements This work has received financial support from the Agencia Española de Cooperación Internacional (A/2385/05) and the Agencia Andaluza de Cooperación Internacional (AI29/04).

References

Adam R (2001) Biology of *Giardia lamblia*. Clin Microbiol Rev 14:447–475

Astiazaran H, Espinosa M, Castanon G, Chavez-Munguia G, Martínez-Palomo A (2000) *Giardia lamblia*: effect of infection with symptomatic and asiymptomatic isolates on the growth of gerbils (*Meriones unguiculatus*). Exp Parasitol 95:128–135

Berkman DS, Lescano AG, Gilman RH, Lopez LS, Black MM (2002) Effects of stunting, diarrhoeal disease, and parasitic infection during infancy oncognition in late childhood: a folow-up study. Lancet 358:564–571

CDC (1995) Division of Parasitic Diseases Program Review. National Center for Infectious Diseases, Centers for Disease Control, Atlanta, G.A. USA, S.C

Cordova O, Vargas F, Gonzalez A, Peréz Cordón G, Velasco JR, Sánchez-Moreno M, Rodríguez Gonzalez I, Rosales MJ (2006) Intestinal parasitism in Peruvian children and molecular characterization of *Cryptosporidium* species. Parasitol Res 98:576–581

Gilman RH, Marquis GS, Miranda E, Vestegui M, Martinez H (1988) Rapid reinfection by *Giardia lamblia* after treatment in a hyperendemic Third World community. Lancet 13:343–345

Gonzales-Mugaburu L (1956) Encuesta de parásitos intestinales en 615 escolares del Rímac. Rev Peru Med Exp Salud Pública 10:2

Haque R, Roy S, Kabir M, Stroup SE, Mondal D, Houpt ER (2005) *Giardia* assemblage A infection and diarrhea in Bangladesh. J Infect Dis 192:2171–2173

Iannacone J, Benites MJ, Chirinos L (2006) Prevalencia de infección por parásitos intestinales en escolares de primaria de Santiago de Surco, Lima, Perú. Parasitol Latinoam 61:54–62

Maco V, Marcos LA, Terashima A, Samalvides F, Gotuzzo E (2002) Distribution of entero-parasitic infections in the Peruvian highland: study carried out in six rural communities of the department of Puno, Peru. Rev Gastroenterol Peru 22:304–309

Monis PT, Thompson RC (2003) *Cryptosporidium* and *Giardia*-zoonoses: fact or fiction? Infect Genet Evol 3:233–244

Monis PT, Mayrhofer G, Andrews RH, Homan WL, Limper L, Ey PL (1996) Molecular genetic analysis of *Giardia intestinalis* isolates at the glutamate dehydrogenase locus. Parasitology 112:1–12

PAHO (1998) Situación de Salud de los Pueblos Indígenas de Perú of the Iniciativa de Salud de los Pueblos Indígenas de OPS. Washington, D.C. p 38

Read CM, Monis PT, Thompson RCA (2004) Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. Infection, Genetics and Evolution 4:125–130

Savioli L, Bundy D, Tomkins A (1992) Intestinal parasitic infections: A soluble public health problem. Trans R Soc Trop Med Hyg 86:353–354

Sulaiman IM, Fayer R, Bern C, Gilman RH, Trour JM, Schantz PM, Das P, Lal A, Xiao L (2003) Triosephosphate somerase gene characterization and potential zoonotic transmission of *Giardia duodenalis*. Emerg Infect Dis 9:1444–1452

Thompson RCA (2000) Giardiasis as a re-emerging infectious disease and its zoonotic potential. Int J Parasitol 30:1259–1267

Thompson RCA (2004) The zoonotic significance and molecular epidemiology of *Giardia* and giardiasis. Vet Parasitol 126:15–35

Thompson RCA, Hopkins RM, Homan WL (2000) Nomenclature and genetic groupings of *Giardia* infecting mammals. Parasitol Today 16:210–213

Torres-Portugal M, Campos E (1960) Encuesta de parasitosis intestinal en niños de edad preescolar en la ciudad de Arequipa.

Revista Peruana de Medicina Experimental y Salud Pública 13:1–2
Vargas F, Jara D, Castillo R (1987) Protozoarios y helmintos intestinales en pobladores de Trujillo, Perú. Rebiol 6:3–14
Volatao AC, Costa-Macedo LM, Haddad FSM, Brandao A, Peralta JM, Fernández O (2007) Genotyping of *Giardia duodenalis* from human and animal simples from Brazil using b-giardin gene: A phylogenetic analysis. Acta Tropica 102:10–19
WHO (1991) Basic laboratory methods in medical parasitology. World Health Organization, Geneva
WHO (1996) Fighting disease, fostering development. World Health Organization, Geneva