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Risk factors for *Giardia* infection among hospitalized children in Cuba

J. BELLO*, F. A. NÚÑEZ†, O. M. GONZÁLEZ‡, R. FERNÁNDEZ*, P. ALMIRALL§ and A. A. ESCOBEDO¶

*Academic Paediatric Hospital of Cerro, Calzada del Cerro No. 2002, Cerro, Ciudad de La Habana, Cuba
†Institute 'Pedro Kourí', Autopista Novia del Mediodía Km 6.5, La Lisa, Ciudad de La Habana, CP 11400, Cuba
‡National Centre for the Coordination of Clinical Trials, Calle 200 y Avenida 19, Playa, Ciudad de La Habana, CP 11600, Cuba
§Faculty of Medicine 'Comandante Manuel Fajardo', Calle D esquina a Zapata, Vedado, Ciudad de La Habana, CP 10400, Cuba
¶Academic Paediatric Hospital 'Pedro Borrás', Calle F No. 616 esquina 27, Vedado, Ciudad de La Habana, CP 10400, Cuba

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The risk factors associated with *Giardia* infection, in children hospitalized in Havana, Cuba, were recently explored. Children aged ⩾5 years were more likely to be positive for *Giardia* infection than the younger children, with an odds ratio (OR) of 3.41 [95% confidence interval (CI)=1.36–9.69]. The risk factors found to be associated with *Giardia* infection in univariate analyses were rural residence (OR=3.01; CI=1.23–7.35), belonging to a household that did not receive water from an aqueduct (OR=3.27; CI=1.21–8.91), drinking unboiled water (OR=3.64; CI=2.14–6.26), nail biting (OR=3.47; CI=1.97–6.08), eating unwashed vegetables raw (OR=4.84; CI=2.33–10.14), and a personal (OR=3.23; CI=1.58–6.59) or family history (OR=3.96; CI=1.53–10.47) of previous parasitic infection. In multivariate analyses, however, only two (modifiable) risk factors were found to be independently and significantly associated with *Giardia* infection: nail biting and eating unwashed vegetables raw. It therefore seems that, at least at the individual level, giardiasis-prevention activities in Havana should be focussed on health education to improve personal hygiene and food-related practices. If appropriately managed, the surveillance of drinking water and foodstuffs, for *Giardia* and other parasites, might also help to reduce the hospitalization of Cuban children.

Acute gastro-intestinal illness represents a substantial burden for the public health system of Cuba, where the risk of such illness is significantly higher among children than in adults (Aguiar Prieto *et al.*, 2009). Diarrhoea, specifically, remains an important cause of illness among infants and young children despite efforts to improve sanitary conditions, water quality and the health infrastructure, the aggressive promotion of oral rehydration therapy and breast-feeding, and general health promotion. Prevention and treatment strategies need to be better targeted against the common agents causing severe diarrhoea and gastro-intestinal discomfort.

Giardia lamblia, the aetiological agent of human giardiasis, is one of the commonest protozoan pathogens associated with infectious childhood parasitic diarrhoea and other gastro-intestinal symptoms (i.e. abdominal pain, flatulence and weight loss),

Reprint requests to: A. A. Escobedo.
E-mail: escobedo@infomed.sld.cu.

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in both industrialized and developing countries (Upcroft and Upcroft, 2002). In developing countries, it has been estimated that 3000 million people live in unsewered environments and that about a third of such people (i.e. approximately 1000 million individuals) have *Giardia* infections at any one time, such infections contributing to the 2.5 million deaths that occur annually as the result of diarrhoeal diseases (Upcroft and Upcroft, 2002).

In their case–control study, conducted in 1999, Núñez *et al.* (2003*a*) searched for the parasitic causes of diarrhoea in children admitted to a Cuban hospital. Although they found *Giardia* in 4.6% of their 'cases' (patients admitted to diarrhoea wards), they found the same parasite in a similar percentage of their 'controls' (patients admitted to other wards).

Although *Giardia* infections are relatively easy to diagnose and curable even with a single oral dose of some antiparasitic drugs (e.g. tinidazole or secnidazole), their early detection and treatment remain important components of efforts to reduce the burden of giardiasis. The identification of risk factors for *Giardia* infection — especially those that are potentially modifiable — may clearly help in developing effective preventive activities. Such risk factors vary with the setting and population involved. Those identified in earlier investigations include: age (of children; Pereira *et al.*, 2007; Coles *et al.*, 2009); number of children in the household, poor food hygiene, day-care-centre attendance, and/or living on a rural farm within the 6 months prior to hospitalization (Pereira *et al.*, 2007); drinking piped water and/or eating raw vegetables (Mohammed Mahdy *et al.*, 2008); living in households without piped water, storing water in jars, cisterns, tanks or buckets, disposing of sewage in septic tanks or directly on the soil, bathing outside the dwelling, and/or poor food hygiene (Cifuentes *et al.*, 2004); lack of an indoor toilet (Prado *et al.*, 2003; Teixeira *et al.*, 2007); living in a house without access to a sewerage system (Ribeiro Silva *et al.*, 2009); eating raw vegetables, male gender and/or impaired immunity (Espelage *et al.*, 2010); and exposure to nappies (Hoque *et al.*, 2001, 2003).

In Cuba, *Giardia* infection in children has been widely reported (Núñez *et al.*, 1999, 2003*b*; Mendoza *et al.*, 2001, 2003; Escobedo *et al.*, 2007) but data on the epidemiology of, and disease burden posed by, symptomatic giardiasis in paediatric patients is scarce. The aim of the present study was to identify the risk factors associated with *Giardia* infection in hospitalized children in the city of Havana. Such information could be relevant in epidemiological surveillance and the planning and implementation of control strategies.

PATIENTS AND METHODS

Study Setting

The study was based at the Academic Paediatric Hospital of Cerro (APHC) in Havana City, Cuba. This 126-bed hospital provides in-patient and out-patient medical services, mainly to the children living in the municipality of Cerro, and admits about 4000 children/year. Healthcare facilities for the children of Cerro include this regional hospital, four smaller health centres (polyclinics) and about 20 dispensaries. Dispensary staff treat uncomplicated diarrhoea (among other illnesses) and refer severe cases to a health centre or the hospital.

Study Design and Enrolment

The study was based on a case–control design, with a 'case' being a child aged <17 years when he or she was admitted, to the APHC in the first 6 months of 2003, with or without diarrhoea (defined as a change in bowel habits, including an increase in the frequency of defecation, an increase in faecal volume or a change in stool consistency — as determined by the patient, if an adolescent, or by the parents/

caregivers of younger children) but with *Giardia* infection (confirmed by the microscopical detection of trophozoites and/or cysts in a faecal sample). A 'control' was a child aged <17 years who was admitted to a general medical ward, of the same hospital during the same period of time, and found *Giardia*-free (but possibly positive for another intestinal parasite) when a faecal sample had been examined.

Sample Size

It was estimated that, with a power (l-β) of 80% and a precision of 95%, and assuming ⩾13% of the subjects would have been exposed to *Giardia* infection, at least 94 cases and 247 controls would be necessary to give odds ratios (OR) for risk factors of ⩾2.5. During the study, 257 controls were enrolled to allow for 10 possible drop-outs, the number of controls enrolled from each general medical ward being proportional to the rate of admission to that ward.

Procedures

During the hospitalization of each subject, one of his or her parents/caregivers was given three labelled plastic vials containing 10% formalin solution and was requested to collect three faecal samples from the subject over a 1-week period. All the faecal samples were transported to the intestinal-parasite laboratory of the Institute of Tropical Medicine 'Pedro Kourí' in Havana City, where each was examined microscopically for parasites, as a direct wet mount, after concentration by Ritchie's method, and as a dry smear stained (for intestinal coccidia) using the modified Ziehl–Neelsen technique (García and Bruckner, 1993).

The nutritional status of each enrolled child was determined from his or her age, weight and height, with comparison to Cuban growth references (Jordan, 1988).

A standardized questionnaire was used, at admission, by the members of the research team, to collect relevant information from at least one of the parents/caregivers of each enrolled child. The data collected related to the child's age, gender, place of residence, attendance at an educational centre, source of drinking water, personal-hygiene habits and clinical features (on presentation at the hospital), the household's toilet facilities, and prior parasitic infections affecting the child and/or his or her immediate family.

Ethical Approval

This study protocol was reviewed and approved by the APHC's Ethics Committee and the Review Board of the Institute of Tropical Medicine 'Pedro Kourí'. Informed consent was obtained not only from a parent/caregiver of each enrolled child but also from each enrolled child aged 7–16 years.

Data Management and Statistical Analysis

All data derived from the questionnaires and parasitological examinations were entered and analysed in version 6 of the Epi Info™ software package (Centers for Disease Control and Prevention, Atlanta, GA), with χ^2 and Fisher's exact tests used, as appropriate, to make inter-group comparisons. Odds ratios (OR) and their 95% confidence intervals (CI) were estimated for each potential risk factor. Each of the variables found to be significantly associated with *Giardia* infection in univariate analyses was entered into a multivariate analysis model, using version 10 of the STATA package (Stata Corporation, College Station, TX). A P-value of <0.05 was considered indicative of a statistically significant difference or association.

RESULTS

Overall, 351 hospitalized children (94 cases, 257 controls, 210 boys and 141 girls) aged 0–14 years were enrolled and three faecal samples were collected from each of them.

In the univariate analysis (see Table 1), children aged ⩾5 years appeared at greater risk of *Giardia* infection than the younger

TABLE 1. *Results of the univariate analyses of the potential risk factors for* Giardia *infection, based on data from 351 paediatric in-patients (94 of whom had* Giardia *infections)*

	No. and (%) of children with characteristic who were:			
Characteristic	Investigated	*Giardia*-positive	*Giardia*-negative	Odds ratio and (95% confidence interval)
AGE (years)				
<1	51	7 (13.7)	44 (86.2)	Reference
1–4	175	43 (24.6)	132 (75.4)	2.05 (0.83–5.77)
⩾5	125	44 (35.2)	81 (64.8)	3.41 (1.36–9.69)
GENDER				
Male	210	52 (24.8)	158 (75.2)	1.29 (0.78–2.14)
Female	141	42 (29.8)	99 (70.2)	
EDUCATIONAL CENTRE ATTENDED				
Day-care centre	143	38 (26.6)	105 (73.4)	Reference
Primary school	86	29 (33.7)	57 (66.3)	1.41 (0.75–2.61)
Secondary school	29	11 (37.9)	18 (62.1)	1.69 (0.66–4.17)
RESIDENCE				
Rural	26	13 (50.0)	13 (50.0)	3.01 (1.23–7.35)*
Urban	325	81 (24.9)	244 (75.1)	
SOURCE OF WATER				
Non-aqueduct	21	11 (52.4)	10 (47.6)	3.27 (1.21–8.91)*
Aqueduct	330	83 (25.2)	247 (74.8)	
FAECAL DISPOSAL				
Latrine in house	19	9 (47.4)	10 (52.6)	2.62 (0.90–7.41)
Toilet	332	85 (25.6)	247 (74.4)	
DRINKS UNBOILED TAP WATER				
Yes	163	65 (39.9)	98 (60.1)	3.64 (2.14–6.26)*
No	188	29 (15.4)	159 (84.6)	
BITES NAILS				
Yes	80	38 (47.5)	42 (52.5)	3.47 (1.97–6.08)*
No	271	56 (20.7)	215 (79.3)	
EATS UNWASHED VEGETABLES RAW				
Yes	41	24 (58.5)	17 (41.5)	4.84 (2.33–10.14)*
No	310	70 (22.6)	240 (77.4)	
PERSONAL HISTORY OF PREVIOUS PARASITIC INFECTION				
Yes	42	21 (50.0)	21 (50.0)	3.23 (1.58–6.59)*
No	309	73 (23.6)	236 (76.4)	
FAMILY HISTORY OF PREVIOUS PARASITIC INFECTION				
Yes	23	13 (56.5)	10 (43.5)	3.96 (1.53–10.47)*
No	328	81 (24.7)	247 (75.3)	

*Statistically significant ($P<0.05$).

children but gender had no marked effect on risk. The children who lived in a rural area appeared to be at a 3.1-fold greater risk of *Giardia* infection than their urban counterparts. Similarly, children from households that did not receive water from an aqueduct were also at relatively high risk of *Giardia* infection, as were those who drank unboiled tap water, those who bit their nails, and those who ate unwashed vegetables raw. Compared with other children, those who already had a personal history of parasitic infection had a 23% increased likelihood of having a current *Giardia* infection, and those who had a family history of parasitic infection had a 96% increased likelihood of current *Giardia* infection.

In the multivariate analysis, however, only nail biting and eating unwashed vegetables raw were found to be independently and significantly associated with *Giardia* infection (see Table 2).

DISCUSSION

In the present study, the children aged ⩾5 years were more likely to be found positive for *Giardia* than the younger subjects. Elsewhere in Cuba, *Giardia* positivity has generally been found to decrease with age, peaking in children aged 2–3 years and decreasing thereafter (Núñez *et al.*, 1999). In most endemic settings, where children acquire primary infections with *Giardia* early in their lives and subsequently improve their personal hygiene, the prevalence of *Giardia* infection might be expected to fall from infanthood (Escobedo *et al.*, 2011). Infection does not necessarily translate into symptomatic disease, however, and the probability that a *Giardia* infection becomes symptomatic may also be age-related. The present study cohort, being hospital-based, was probably biased towards children showing the more severe consequences of *Giardia* infection (see below). In Brazil, Ribeiro Silva *et al.* (2009) simply found an age of ⩾2 years to be a risk factor for *Giardia* infection, and Pereira *et al.* (2007), working with children hospitalized for diarrhoea, found that the odds of *Giardia* infection increased about 1.18-fold for each additional year of age. In another Brazilian study (Teixeira *et al.*, 2007) and in an investigation in Turkey (Okyay *et al.*, 2004), however, age appeared to have no significant effect on the risk of *Giardia* infection.

Infection seemed to occur equally commonly among the girls and boys investigated in the present study. Although similar observations have been made in Brazil (Pereira *et al.*, 2007) and Cuban day-care centres (Núñez *et al.*, 1999), boys appeared at higher risk of *Giardia* infection than girls in investigations in the U.S.A. (Yoder and Beach, 2007), Germany (Espelage *et al.*, 2010) and in a recent study in another hospital in Havana (Escobedo *et al.*, 2011).

For a better understanding of giardiasis, there is a need to investigate the risk factors for the disease and, perhaps particularly, the importance, especially among children, of good personal hygiene in the prevention of infection. The epidemiology of *Giardia* infection is greatly influenced by certain factors that facilitate the dispersion of human faeces in the environment, and the subsequent contamination of water and food with the parasite's cysts. In the present study, even in the multivariate analysis, *Giardia* infection was found to be significantly associated with the eating of raw, unwashed vegetables. In Cuba, in their longitudinal study based in three day-care centres in Havana City, Núñez *et al.* (1999) identified a group of children who were at relatively high risk of being re-infected with *Giardia*, and this high risk was subsequently linked to the drinking of unboiled tap water

TABLE 2. *Results of the multivariate analyses of the potential risk factors for* Giardia *infection, based on data from 351 paediatric in-patients (94 of whom had* Giardia *infections)*

Potential risk factor	Odds ratio and (95% confidence interval)	S.E.	*z*	*P*
Residence	0.7 (0.0–5.9)	0.63	0.33	0.740
Source of water	2.3 (0.2–23.7)	1.99	−0.72	0.469
Drinking unboiled tap water	1.1 (0.7–1.6)	5.93	−0.55	0.579
Nail biting	3.2 (1.8–5.7)	11.13	−4.04	<0.001*
Eating unwashed vegetables raw	2.9 (1.4–6.2)	7.76	−2.85	0.004*
Personal history of parasitic infection	1.7 (0.7–3.8)	4.05	−1.26	0.206
Family history of parasitic infection	2.2 (0.7–6.2)	4.21	−1.51	0.130

*Statistically significant ($P<0.05$).

and (in the children's households) poor hygiene during the preparation of vegetables (Núñez *et al.*, 2003*b*). In Malaysia, similarly, Mohammed Mahdy *et al.* (2008) found the eating of raw vegetables to be significantly associated with the risk of giardiasis, and the results of a case–control study in the U.K. (Stuart *et al.*, 2003) also indicated that the eating of green salad (i.e. raw vegetables) was positively and independently associated with *Giardia* infection. Given the potential for food- and water-borne giardiasis — itself a consequence of the very small size and environmental robustness of *Giardia* cysts, the very high numbers of such cysts shed by some infected individuals, and the very low dose of cysts required to initiate a human infection (Escobedo *et al.*, 2010) — it is easy to understand the impact that food-handling hygiene may have on *Giardia* transmission. Vegetables and fruit may be contaminated with *Giardia* cysts, in the field, during chain irrigation and fertilization activities (Amorós *et al.*, 2010). Cysts may also be transferred to foodstuffs, within packing sheds and households, directly from individuals who are shedding cysts. Irrigation water and wash water used in the fresh-produce industry can contain *Giardia* cysts that may contaminate fruit or vegetables (Robertson and Gjerde, 2001; Thurston-Enriquez *et al.*, 2002; Chaidez *et al.*, 2004; Lonigro *et al.*, 2006; Robertson, 2007; Vuong *et al.*, 2007). Such cysts have been detected on water spinach, lettuce, various herbs, strawberries, sprouted seeds, potatoes, carrots, and fresh coriander (Amahmid *et al.*, 1999; Takayanagui *et al.*, 2000; Robertson and Gjerde, 2001; Vuong *et al.*, 2007). In the U.S.A., outbreaks of giardiasis have been attributed to eating salad contaminated by food handlers (Rose and Slifko, 1999). Hygienic food-handling procedures can help prevent *Giardia* infection from spreading, indirectly, from person-to-person, as well as the introduction of contaminated food into households.

The association observed, in the present study, between nail biting and *Giardia* infection was not surprising, given that young children have poor standards of personal hygiene, are generally gregarious, share toys that may be contaminated with faeces, and have high levels of hand–mouth contact. The children investigated by Schuman (1983) took their hands or a loose object to their mouths at a mean frequency of 20 times/h.

The value of the results presented here is reduced by several limitations of the present study. Firstly, the enrolment period was only 6 months and, in consequence, the number of cases identified was fairly small. Secondly, it was hospital-based and restricted to in-patients who probably had the more severe manifestations of *Giardia* infection. Thirdly, as the results only show statistical associations between *Giardia* infection and certain of the factors that were investigated, valid cause–effect inferences cannot be made. Finally, the identification of the causes of diarrhoea episodes was limited by the assays performed (non-*Giardia* causes, such as bacteria and viruses, could not be excluded since there was no appropriate testing). Despite these limitations, however, the present results indicate that many *Giardia*-attributable hospitalizations could be prevented without applying extraordinary input. Health education to improve food and personal hygiene (perhaps focussed simply on increasing hand washing) and the routine surveillance, for pathogenic parasites, of fruit and vegetables that are often eaten raw and of water are recommended. Further studies are needed to determine the financial implications of *Giardia* infection, and the epidemiology and burden of such infection at community and national levels.

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