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Risk factors for infection with *Giardia duodenalis* in pre-school children in the city of Salvador, Brazil

M. S. PRADO[1], A. STRINA[1], M. L. BARRETO[1], ANA MARLÚCIA OLIVEIRA-ASSIS[2], LÍVIA MARIA PAZ[1] AND S. CAIRNCROSS[3]*

[1] *Instituto de Saúde Coletiva, Universidade Federal da Bahia, Brazil*
[2] *Escola de Nutrição, Universidade Federal da Bahia, Brazil*
[3] *London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E 7HT, UK*

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SUMMARY

A cross-sectional study of 694 children aged 2 to 45 months selected from 30 clusters throughout the city of Salvador, Bahia (pop. 2·3 million) was carried out as part of a longitudinal study of diarrhoea in order to identify risk factors for infection with *Giardia duodenalis*. Variables studied included three social and demographic factors (such as mother's education and marital status), five relating to the peri-domestic environment (rubbish disposal, open sewers, paving of the street), seven relating to the home itself (house construction, susceptibility to flooding, water supply and sanitation) as well as a score for hygiene behaviour based on structured observation. After multivariate analysis using a hierarchical model, only four significant risk factors were found: (a) number of children in the household under five years (b) rubbish not collected from the house (c) presence of visible sewage nearby, and (d) absence of a toilet. All four were significant at the 1% level.

INTRODUCTION

Infection with the protozoan enteric pathogen *Giardia duodenalis* is common, particularly among pre-school children, and not only in poor communities in developing countries. It has been estimated that a global total of some 200 million people are infected [1]. The prevalence of infection varies from 2 to 5% in industrialised countries and from 20 to 30% in the developing countries of the world [2–5].

Most infections with *G. duodenalis* are asymptomatic. Among symptomatic children the most important signs are persistent diarrhoea and loss of weight. However, a wide range of other symptoms has also been noted, including nausea, malabsorption of lactose, carbohydrate, fats, and vitamins A and B_{12} [6, 7], macrocytic anaemia due to folate deficiency and retardation of growth and development [8, 9].

Transmission of *G. duodenalis* is by the faeco-oral route, and epidemics, as well as endemic cases in the developed countries, have been associated with waterborne transmission [10]. Person to person transmission has also been documented in institutions such as creches and children's wards where hygiene conditions are less than ideal [11]. The pathogen is found in wild animal reservoirs such as beavers [12], and also in domesticated animals including cattle, cats and dogs [13]. However, most studies of the transmission of this pathogen have investigated epidemic conditions and developed countries, and the environmental epidemiology of endemic giardiasis in developing countries has received relatively little attention [14]. The present study aims to identify environmental risk factors for *G. duodenalis* infection among

* Author for correspondence.

pre-school children in the City of Salvador in North-east Brazil.

METHODS

Study design and population

The study site was the City of Salvador, capital of Bahia State, with a population of approximately 2·3 millions and a population density of 6630 inhabitants/km² [15]. The sample had originally been selected for a wider longitudinal study of the health impact of sanitation. The sampling has been described in detail elsewhere [16]. First, 30 neighbourhoods were selected, using stratified random sampling to represent the range of environmental conditions found throughout the city. The city was divided into areas with differing degrees of coverage with environmental services such as water supply and excreta disposal, using data from the 1991 national census [15] and a random sample of neighbourhoods chosen from each. Each neighbourhood included a mean of 600 contiguous dwellings, occupying one or more census tracts. A census of each neighbourhood was conducted, giving a listing of all households with children aged from 0 to 3 years, based on the mother's declaration of the child's age. A subset of these households was then chosen at random. In households with more than one eligible child, one such child was also selected randomly to be recruited to the study. The collection of stool samples usually took place 6 months (occasionally up to 9 months) after the selection of the original sample. Thus the children were aged up to 45 months at the time of the study.

Collection and examination of stool samples

A numbered, sealable container was given to the mother or carer of each child at home and she was asked to collect a stool sample the following morning. These were collected the next morning and immediately transported under refrigeration to the laboratory for examination on the same day. If the sample was too small or the child's family did not present a sample, the field worker arranged to collect a new sample on the following day. A single stool sample from each child was examined using the spontaneous sedimentation technique, and was considered positive if *G. duodenalis* cysts were found in the sediment [17].

All children found to be infected were treated with 1·5 ml/kg metronidazole (Flagyl®). Due to operational delays, this took place between 1 and 2 months after collection of the samples.

Socio-economic and environmental data

Socio-economic and environmental data were collected at the time of recruitment to the study, using a pre-coded questionnaire and observation schedule. The observation schedule included provision for the field workers to note particularly hygienic or unhygienic behaviour by the child and her mother occurring during their visits, which were made twice a week for a year. A composite score was composed of 33 different behaviours, and children were grouped into three categories; those for whom the observed behaviours were mainly unhygienic, those in which hygienic and unhygienic behaviours were observed with roughly equal frequency, and those in which unhygienic behaviour was most commonly observed. Details of the hygiene behaviour observation are given elsewhere [18]. The field workers were all females, with full secondary education. They were selected on the basis of a simulated interview using the study questionnaire, and given a week's training. The questionnaire itself had been pre-tested in the field. One in ten households was re-interviewed by the supervisor, a trained sociologist, as a quality control measure.

The variables studied fell broadly into four categories: (a) those related to the socio-economic and demographic status of the mother and the household; (b) those reflecting the characteristics of the peri-domestic environment; (c) environmental characteristics of the household itself; and (d) hygiene-related behaviour of the mother and her child (see Table 1).

Statistical analysis

Statistical analysis was carried out using STATA, version 7.0. After bivariate analysis consisting of prevalence ratios (PR) and 95% confidence intervals (CI), the following procedure was used for multivariate logistical regression. The various explanatory variables were grouped in a hierarchical model of the causation of infection [19], with the groups as shown in Table 1. The first stage was to construct a multivariate model including only the socio-economic and demographic variables. All the variables in this group except sex were included, whether or not they were

Table 1. *Potential risk factors for* Giardia duodenalis *infection included in the study*

Group	Variables studied
(a) Socio-economic, demographic	Age and sex of child
	Number of children <5 years in household
	Mother's education and marital status
(b) Peri-domestic environment	Whether rubbish collected from house
	Frequency of rubbish collection
	Presence of rubbish heap near house
	Presence of visible sewage near house
	Paving of street
(c) Domestic environment	House construction (permanent/shack)
	House floor (earth or planks/cement)
	Susceptibility to flooding during rain
	Piped water supply in house
	Intermittence of water supply
	Presence/absence of a toilet
	Whether drinking water boiled
(d) Mother's/child's hygiene behaviour	Composite score (mainly positive/neutral/mainly negative)

significant in the bivariate analysis. Those which did not show an association significant at the 5% level were removed from the model one by one, the least significant first. Then all the variables in group (b) (see Table 1) were added to the model, and the variables without a significant association were removed one by one, as before. The process was then repeated with group (c) and finally group (d). The results were expressed in terms of odds ratios.

Ethics

Informed consent to participate in the study was obtained from all study households. Ethical approval for the study was given by the Ethics Review Board of the Federal University of Bahia.

RESULTS

Out of a total of 1156 children enrolled in the longitudinal study, stool samples were successfully collected for 694 (60·0%). Of these, 95 (13·7%) were infected with *G. duodenalis*.

Table 2 shows the results of bivariate analysis of socio-economic and demographic risk factors. The prevalence of infection was slightly lower among children aged less than two years, but there was no statistically significant association with age or with sex. Some association was seen with the mother's marital status, although this was not significant. On the other hand, *G. duodenalis* infection showed a significant association with the number of children in the household under 5 years, and also with the mother's level of schooling.

All of the peridomestic environmental variables showed a statistically significant association with *G. duodenalis* infection (Table 3), but there was a high degree of association between them. With regard to the domestic environmental variables (Table 4), significant associations with infection were only found with house type and absence of a toilet in the house, although the lack of significance of some other variables may be attributable to the small numbers exposed to them. The prevalence of giardiasis was in fact lower in households reporting that they boiled or filtered their drinking water, though this association also was not statistically significant.

The unadjusted odds ratio for the hygiene behaviour score (Table 5) shows that children whose mothers were observed to be not particularly careful in the preparation and handling of foodstuffs, milk bottle, comforter and utensils had nearly twice the odds of *G. duodenalis* infection compared to those whose mothers showed particularly hygienic behaviour. However, the association between hygiene behaviour and the prevalence of giardiasis was not statistically significant (OR = 1·76; CI 0·83–3·73).

After construction of a multivariate logistical regression model as described above, only four potential risk factors remained as significant determinants

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Table 2. *Demographic and socio-economic risk factors for infection with* G. duodenalis *among pre-school children, Salvador, Brazil 1998*

Variables	*N*	% positive	OR (95% CI)
Demographic factors			
Age			
12–23 months	294	12·9	—
24–35 months	231	14·3	1·22 (0·68–1·85)
35–45 months	169	14·2	1·11 (0·64–1·93)
Sex			
Male	376	13·6	—
Female	318	13·8	1·02 (0·66–1·58)
No. of children in family <5 years			
1	467	10·1	—
>1	227	21·1	2·39 (1·55–3·72)
Socio-economic factors			
Mother's marital status			
Married	216	17·6	—
Other	478	11·9	0·63 (0·41–0·99)
Mother's education			
⩾8 years	241	9·1	—
<8 years	453	16·1	1·91 (1·15–3·17)

Table 3. *Peridomestic environmental risk factors for infection with* G. duodenalis *among pre-school children in Salvador, Brazil 1998*

Variables	*N*	% positive	OR (95% CI)
Peridomestic factors			
Solid waste disposal			
Collected from house	375	8·8	—
Dumped	317	19·6	2·52 (1·60–3·97)
Frequency of solid waste collection			
Regular	502	10·6	—
Irregular	192	21·9	2·37 (1·52–3·70)
Presence of rubbish heap near house			
Not present	339	9·7	—
Present	355	17·5	1·96 (1·25–3·08)
Presence of visible sewage near house			
Not present	394	9·4	—
Present	300	19·3	2·31 (1·48–3·60)
Condition of street or pathway			
Paved	334	9·9	—
Unpaved	360	17·2	1·89 (1·21–2·98)

of *G. duodenalis* infection. These were: (a) number of children less than 5 years old in the household, (b) rubbish not collected from the house (c) presence of visible sewage near the house, and (d) non-possession of a toilet. The odds ratios and 95% confidence intervals for these are shown in Table 6.

DISCUSSION

In some ways, the present study is complementary to the study by Newman et al. [20] also conducted in urban Northeast Brazil. While the latter examined the factors associated with symptoms among children

Table 4. *Domestic environmental risk factors for infection with* G. duodenalis *among pre-school children in Salvador, Brazil 1998*

Variables	*N*	% positive	OR (95% CI)
Domestic environmental factors			
Type of house			
Permanent construction	637	12·9	—
Shack	57	22·8	2·0 (1·03–3·87)
Floor material			
Cement	655	13·4	—
Earth or planks	38	18·4	1·46 (0·62–3·41)
Separate kitchen			
Yes	509	13·6	—
No	184	14·1	1·05 (0·65–1·70)
House floods during rain			
No	547	13·3	—
Yes	147	15·0	1·14 (0·68–1·91)
Water supply			
Piped connection	604	12·9	—
No piped connection	90	18·9	1·57 (0·88–2·80)
Reliability of water supply			
Regular	472	13·1	—
Intermittent	222	14·9	1·16 (0·73–1·82)
Domestic water storage vessel			
Adequate	475	12·8	—
Inadequate	219	15·5	1·25 (0·79–1·96)
Presence/absence of a toilet			
Present	635	12·1	—
Not present	59	30·5	3·18 (1·74–5·82)
Drinking water boiled or filtered			
Yes	118	18·6	—
No	576	12·7	0·63 (0·38–1·07)

Table 5. *Hygiene behaviour score as a risk factor for infection with* G. duodenalis *among pre-school children in Salvador, Brazil 1998*

Variable	*N*	% positive	OR (95% CI)
Hygiene behaviour			
Mainly positive	165	10·3	—
Neutral	419	15·0	1·54 (0·87–2·72)
Mainly negative	89	15·8	1·76 (0·83–3·73)

who were already infected with *G. duodenalis*, our study examined the risk factors for infection, whether or not it was symptomatic. The overall prevalence of infection (13·6%) found in our study of children aged 12–45 months was slightly higher than that reported by Newman et al. (8·8%). This is understandable as the latter followed up children from birth, although most children were aged over 12 months before their first infection was detected.

The absence, in our data, of an association with any of the three variables related to water supply is striking in view of the studies from industrialised countries which have underlined the role of water in transmission. Studies in rural Africa [14] and urban Brazil [21] have found no significant association between *G. duodenalis* infection and the quality of the water used by the household for drinking. A low quantity of water used for hygiene was significantly associated with giardiasis in the former [14], but in the latter [21] the presence of a piped water connection, normally associated with substantial improvements in hygiene, was not. Indeed in a third study, in Colombia [22], piped water was found to be associated with a *greater* risk of infection.

The Brazilian study mentioned above [21] found [on bivariate analysis] that, giardiasis was associated with low socio-economic status (represented by household income and parents' education). We also found an association with the mother's education on

Table 6. *Risk factors for infection with* Giardia duodenalis *in Salvador, Brazil; results of multivariate logistical regression*

Variable	Odds ratio	*P* value	95% CI
No. of children in family <5 years	2·08	0·001	(1·32–3·27)
Solid waste disposal	1·97	0·005	(1·22–3·16)
Presence of visible sewage near house	1·85	0·009	(1·16–2·96)
Absence of a toilet	2·51	0·004	(1·33–4·71)

bivariate analysis, but this association was no longer significant when controlled for other variables using multivariate analysis.

Another variable, striking by its absence from the final list of risk factors, was the hygiene behaviour score, derived from structured observations made during the home visits. In a study of diarrhoea symptoms in the same group of children [18] the association with this hygiene score was significant and stronger than for all other risk factors, supporting the view that the score was a genuine measure of hygiene standards.

More generally, the differences between the results reported here and those of that diarrhoea study suggest that the transmission of giardiasis is subject to different factors, and may even follow different routes, than the transmission of other diarrhoea pathogens. This conclusion is also supported by the finding of high prevalences of giardiasis in children in day-care centres in the developed countries, although the incidence of diarrhoea among them is not high [23].

Of the risk factors whose statistical significance remains following multivariate analysis, the number of children in the household under five years of age is easily understood in terms of the higher prevalence of infection among young children [21] and the likelihood of transmission between children within the domestic domain. The number of young children in the household is often associated with crowding (persons/room), and this may be the reason why crowding appeared as a risk factor for symptomatic giardiasis in the results of Newman et al. [20].

The second and third significant risk factors – lack of rubbish collection, and visible sewage near the house – are related to peridomestic conditions rather than to hygiene within the home. This fits with the finding that giardiasis is often more prevalent among children over 2 years than the youngest toddlers [14], as the latter are less likely to be allowed to wander into the neighbourhood. Certainly it would help to explain why domestic hygiene behaviour was not significantly protective from *G. duodenalis* infection, if most of the transmission occurs in the public and not the domestic domain [24].

The presence of an open sewer near the house has been found to be associated with infection of Brazilian children with other faecal pathogens [25, 26]. Exposure to open sewers can be seen as analogous to exposure to untreated wastewater used for irrigation [27] although, in the latter, the associated risk seems to stem from the consumption of the irrigated vegetables rather than from wastewater contact [28]. Children are more likely to have contact with wastewater exposed in the peridomestic environment than in the fields.

A number of studies have found an excess risk of diarrhoeal disease associated with deficient solid waste management near the home [29]. Domestic refuse in Brazil contains a substantial amount of faecal contamination, as 5% of it consists of used toilet paper and disposable nappies, even in low-income areas [30]. There are several possible mechanisms by which refuse could promote the transmission of *G. duodenalis* to children. Children can be seen playing on rubbish heaps near their homes, so that direct contact is the most obvious of these. In addition, rubbish in the residential environment may breed houseflies, which have been shown to transmit a quarter of endemic diarrhoea in some communities [31]. However, similar proportions of mothers in households with and without an infected child complained of fly problems (33/92 *vs.* 157/536; RR = 1·29, CI 0·87–1·91), so that this seems unlikely to explain the association that we found. The third mechanism is that rubbish heaps may also attract stray dogs and rats, which are themselves infected with *G. duodenalis* [32].

The fourth risk factor – possession of a toilet – has been found to be protective from *G. duodenalis* infection in emergency camps in Colombia [222], but not in rural Africa [14]. The importance of sanitation may be a function of population density. It may also be that ownership of a toilet sometimes appears protective because it reflects higher socio-economic status or greater awareness of hygiene, rather than the hygienic advantages of the toilet *per se* [18].

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