SUPPLEMENT ARTICLE

A Systematic Review and Meta-analysis of the Association Between *Giardia lamblia* and Endemic Pediatric Diarrhea in Developing Countries

Khitam Muhsen and Myron M. Levine

Center for Vaccine Development, University of Maryland School of Medicine, Baltimore

We performed a systematic literature review and meta-analysis examining the association between diarrhea in young children in nonindustrialized settings and *Giardia lamblia* infection. Eligible were case/control and longitudinal studies that defined the outcome as acute or persistent (>14 days) diarrhea, adjusted for confounders and lasting for at least 1 year. Data on *G. lamblia* detection (mainly in stools) from diarrhea patients and controls without diarrhea were abstracted. Random effects model meta-analysis obtained pooled odds ratios (ORs) and 95% confidence intervals (CIs). Twelve nonindustrialized-setting acute pediatric diarrhea studies met the meta-analysis inclusion criteria. Random-effects model meta-analysis of combined results (9774 acute diarrhea cases and 8766 controls) yielded a pooled OR of 0.60 (95% CI, .38–.94; $P = .03$), indicating that *G. lamblia* was not associated with acute diarrhea. However, limited data suggest that initial *Giardia* infections in early infancy may be positively associated with diarrhea. Meta-analysis of 5 persistent diarrhea studies showed a pooled OR of 3.18 (95% CI, 1.50–6.76; $P < .001$), positively linking *Giardia* with that syndrome. The well-powered Global Enteric Multicenter Study (GEMS) is prospectively addressing the association between *G. lamblia* infection and diarrhea in children in developing countries.

Giardia lamblia (synonymous with *Giardia duodenalis* and *Giardia intestinalis*) is a unicellular eukaryotic microscopic enteric protozoa [1–4] that has been incriminated as a cause of diarrhea in individuals in both industrialized and developing countries [5–9]. When clinical illness ensues, it ranges from self-limited acute to persistent diarrhea [4, 10, 11], accompanied by malabsorption. The circumstances under which *G. lamblia* constitutes an etiologic agent of acute or persistent diarrheal disease are not well understood, since in other instances it colonizes without causing diarrhea and in yet other conditions it appears actually to protect against certain forms of diarrheal disease [12, 13].

Experimental challenge studies unequivocally document that some strains of *G. lamblia* can cause diarrhea in healthy adult volunteers [14, 15], and convincing epidemiological descriptions of acute gastroenteritis outbreaks also provide evidence that in certain hosts and settings this protozoan causes acute diarrhea [11, 16–23]. Finally, some case/control studies and longitudinal studies that prospectively follow cohorts of children (and occasionally adults) also support the notion that *G. lamblia* infection is associated with acute or persistent diarrhea [6, 7, 24, 25]. On the other hand, many other case/control and prospective cohort studies do not incriminate *G. lamblia* as a cause of diarrhea [26–28]; moreover, several studies suggest that carriage of this protozoan actually protects against diarrhea [12, 13, 29, 30].

Because of this confusing situation with respect to the role of *G. lamblia* as an enteric pathogen and the

Correspondence: Khitam Muhsen, PhD, Center for Vaccine Development, University of Maryland School of Medicine, 685 W Baltimore St, Baltimore, MD 21201 (e-mail: kmuhsen@medicine.umaryland.edu).

Clinical Infectious Diseases 2012;55(S4):S271–93

DOI: 10.1093/cid/cis762

ensuing clinical and epidemiologic equipoise, we systematically reviewed the literature and performed a meta-analysis to examine the association between the occurrence of diarrheal disease in young children in developing countries and the presence of *G. lamblia* in their stool samples. We hypothesized that the association linking *G. lamblia* with diarrhea may differ whether one examines the clinical syndrome of acute vs persistent diarrhea; we further hypothesized that the association may be age-dependent. Finally, we reviewed the role of *G. lamblia* as a putative cause of traveler's diarrhea (albeit mainly in adults), anticipating that these data might shed additional light on the circumstances under which *G. lamblia* causes diarrhea.

METHODS

We performed a PubMed literature search (limited to English-language publications of human studies published prior to 1 April 2012) using the terms "*Giardia* and diarrhea," "*Giardia* gastroenteritis," "*Giardia* and travelers' diarrhea," and "etiology of travelers' diarrhea." To detect additional relevant publications, we used the PubMed option of "related articles" and checked the reference lists of the original and review articles.

Exclusion Criteria

Studies conducted in developed countries, case reports, case series, and studies conducted in patients with immunodeficiency or immunocompromise (eg, human immunodeficiency virus, cancer, post–organ transplantation) were excluded. Also excluded were cross-sectional studies and descriptive studies on the prevalence or detection proportion of *G. lamblia* in patients with diarrhea if they did not include a comparison/control group of subjects without diarrhea. If more than one report was published from the same study, only one report was included. The epidemiologic studies were critically reviewed with special emphasis on whether methodological limitations were evident.

Data Abstraction and Tabulation

Data on study design, study population, sampling frame and sample size, methods to detect *G. lamblia*, definition of diarrhea, case ascertainment, results, and matching or adjusting for potential confounders from each study were abstracted onto standardized forms.

Data extracted from the case/control studies included the number of diarrhea patients and control subjects without diarrhea and the number and/or proportion of cases and controls infected with *G. lamblia*. From the cohort studies we abstracted data on the number of stool samples that were obtained during diarrheal episodes, the number of stool samples that were obtained through routine surveillance in the absence of diarrhea, and the number and proportion of diarrheal and nondiarrheal stools that were positive for *G. lamblia*. Alternatively, depending on the design and the analysis in the original study, data were abstracted on the incidence of diarrheal disease in periods that were classified as *G. lamblia* positive or *G. lamblia* negative. Results stratified by age or other variables were abstracted if they were presented in the original articles. From studies that addressed the association between *G. lamblia* genotype and diarrhea, we abstracted data on the number of patients with diarrhea or gastrointestinal symptoms (cases), number of asymptomatic subjects (controls), the number and percentage of cases and controls infected with genotype A, and the number and percentage of the infected cases and controls with genotype B. If data on the rate ratio or odds ratio (OR) and 95% confidence intervals (CIs) or *P* value were presented in the original article, they were abstracted; otherwise we performed the calculations using WinPepi software version 11.15 [31].

Meta-analysis

Meta-analysis was performed to answer the question of whether *G. lamblia* infection is associated with an increased or a decreased risk of endemic diarrheal disease, using data that were generated by case/control or cohort studies. Pooled measurement of association was obtained using the random effects model and forest plots were generated to display summarized results. Heterogeneity among the studies was tested using heterogeneity χ^2 test and I^2 index [32]. Analyses were performed with stratification by the definition of the outcome (acute vs persistent diarrhea). Potential publication bias was assessed using funnel plots with the log OR of each study on the x-axis plotted against its standard error in the y-axis [33]. We also used the Egger regression intercept [34] to detect publication bias and we performed a cumulative meta-analysis (starting with the largest study) to assess the impact of the study size on the direction of the pooled risk estimate. The Comprehensive Meta-Analysis software package (version 2) was used to produce the analyses [35].

Meta-analysis was restricted to studies conducted in developing countries and other resource-poor settings that presented age-specific findings that allowed data abstraction of the results among children. Additional inclusion criteria were demonstration that matching or adjustment for potential confounders (eg, age, sex) was performed, the definition and duration of diarrhea were provided, and the study endured for at least 1 year (to account for seasonality). In the statistical analyses, we used the adjusted effect estimates of each study to obtain a pooled point estimate. However, if no multivariate analysis was conducted, we used the crude risk estimates.

A PRIMER ON *G. LAMBLIA* INFECTION

Because the biology of *G. lamblia* infection has been previously reviewed [2–4, 10, 36–40], only a few salient features are mentioned in this systematic review of the epidemiology.

The Life Cycle of *G. lamblia*

Giardia lamblia, a unicellular eukaryotic flagellated enteric protozoa [1–4] first described by van Leeuwenhoek in 1681 [2–4], occurs as a nonmotile cyst (responsible for transmission) or a motile trophozoite (associated with clinical symptoms) [4, 10]. Low gastric acidity followed by exposure to pancreatic secretions prompts excystation in the proximal small intestine, with 2 trophozoites deriving from each cyst. The trophozoites replicate in the lumen by binary fission and adhere to enterocytes of the proximal small intestine by suction (using their ventral adhesive disk) [1, 3, 4, 10] and by specific receptor-ligand interactions [36], but do not invade the epithelium. Encystation begins in the small intestine upon exposure to bile salts and is promoted by alkaline pH and decreasing cholesterol levels [1, 3, 4, 10, 41]. Both cysts and trophozoites may be excreted, depending on the nature of the stool. *Giardia* cysts survive in the environment for weeks and months [3, 4, 10], especially in cool and moist conditions [5].

Transmission

Giardia lamblia is transmitted via the ingestion of as few as 10 cysts [42]. Much information on the modes of transmission of *Giardia* comes from studies of infection and illness in industrialized-country settings. Waterborne transmission of *G. lamblia* is well documented [17, 20, 21, 43], including through recreational water activities and swimming [44–48]. The low inoculum facilitates person-to-person transmission among family members [18, 22, 49, 50] and subjects in crowded conditions where hygiene practices may be suboptimal (eg, daycare centers) [16, 18, 22]. Foodborne transmission of *G. lamblia* occurs but is uncommon [16, 51, 52]. Sexual transmission has been reported among men who have sex with men [53–56].

Epidemiological studies [57–61] and genotyping studies of *G. lamblia* support the possibility of zoonotic transmission [62, 63] of *G. lamblia* assemblages A and B, genotypes known to infect both humans and other host species; genotypes C to G infect only animals [64].

Giardia Clinical Illness

Analysis of responses of volunteers to ingestion of *G. lamblia* and descriptions of patients with disease consequent to well-described outbreaks attributed to the protozoan show that the main symptoms include diarrhea, abdominal pain, nausea, vomiting, flatulence, anorexia, and fever [4, 10, 11, 14]. In most instances the diarrheal illness is short-lived and self-limited. However, a proportion of individuals develop persistent diarrhea [4, 10, 11, 65], sometimes accompanied by malabsorption of sugars and fat and by weight loss. In both volunteers and outbreak situations, a sizable proportion of the infected subjects are asymptomatic, often exceeding the proportion who manifest clinical illness [65, 66].

THE ASSOCIATION BETWEEN *G. LAMBLIA* AND DIARRHEAL DISEASE

We identified 46 case/control studies and 18 longitudinal studies conducted from the 1970s through 2009 in developing-country and transitional populations that addressed the association between *G. lamblia* and endemic diarrheal disease.

Overview of the Case/Control Studies

Most of the case/control studies addressed the broad etiology or the role of protozoal agents in acute diarrheal disease, with *G. lamblia* being one of multiple enteropathogens looked for in stools [6, 7, 12, 13, 25, 26, 28, 67–103]. Children comprised the target population in the majority of the studies [6, 7, 12, 13, 25, 26, 28, 67, 68, 70–74, 76, 78, 79, 81, 82, 84–86, 88–91, 93–104], although some studies included adults with or without children [69, 75, 77, 80, 83, 87, 92, 105].

Case ascertainment was performed in the community [78, 96], outpatient clinics [6, 25, 28, 69, 73, 76, 81, 82, 91, 97, 102, 103], emergency rooms [83, 90, 95], or hospitals [7, 12, 13, 26, 68, 70–72, 75, 77, 80, 84, 86, 87, 93, 100, 104, 105]. The control subjects without diarrhea were outpatients [6, 13, 25, 28, 67, 69, 72, 73, 76, 80, 81, 89, 91, 95, 97, 101–103] emergency room patients [83], or hospitalized patients [7, 12, 13, 26, 70, 71, 75, 77, 84, 86, 87, 100, 104, 105], but some studies enrolled community controls [68, 78, 82, 93, 96]. Both inpatient and outpatient settings comprised the sampling frame for some studies [67, 74, 79, 85, 92, 98, 101].

Matching (or adjustment for confounders) between cases and controls by age, sex, and other variables was done in only a fraction of the studies [12, 13, 25, 28, 67, 68, 70, 72, 73, 75, 76, 78–80, 82, 83, 85, 86, 88, 90, 91, 93, 96–98, 102, 103, 105]. In others, neither matching nor adjustment for confounding effects in multivariate analyses was performed [6, 7, 26, 71, 74, 77, 81, 84, 87, 89, 92, 94, 95, 99–101, 104].

Some studies proceeded for at least 1 year [6, 7, 12, 13, 25, 28, 68–73, 75, 79, 80, 82, 84, 86, 90, 92–94, 96, 98, 100], while others lasted only a few months. Stool microscopy was the method most often used for detecting *G. lamblia* [6, 7, 12, 13, 25, 26, 28, 67–69, 71–79, 81–88, 90–97, 100–103]. In a few studies enzyme immunoassay [70, 80, 98, 105] or polymerase chain reaction [89] was used, either in addition to microscopy or as the exclusive method, to detect *Giardia* in stools or duodenal aspirates [104].

The outcome variable was "acute diarrhea" in the majority of the studies [26, 28, 68–73, 75, 76, 80–83, 85, 86, 88, 90, 92, 95, 98, 99, 101, 103, 105], but a few included "persistent diarrhea" as well as "acute diarrhea" [7, 25, 84, 97, 102, 104]; some studies presented pooled results of acute and persistent diarrhea. In 14 studies the length of the diarrheal episode was not specified [6, 12, 13, 67, 74, 77–79, 87, 89, 91, 93, 94, 96] or was not clearly defined [100]. A few studies focused on persistent diarrhea as the outcome variable [106–109], defined as diarrhea that continued unabated for >2 weeks; these studies are presented separately. In one study no operational definition was presented and in another study the authors reported on "chronic diarrhea," defined as diarrhea that lasted >4 weeks [104].

Table 1 summarizes salient features of 12 pediatric case/control studies of acute diarrhea [28, 68, 70, 72, 73, 76, 80, 82, 86, 90, 98, 103] and 3 studies of persistent diarrhea [106, 107, 109] in which the authors controlled for potential confounders by matching or adjusting in multivariable analysis. From one study we abstracted data on children only [80].

Acute Diarrhea

Among studies conducted in children in developing countries or other nonindustrialized settings, 6 studies showed no significant difference between cases and controls in the detection rate of *G. lamblia* [28, 73, 76, 82, 90, 103], whereas 6 other studies showed a significantly lower detection rate of *G. lamblia* in stools from patients with acute diarrhea than from controls [68, 70, 72, 80, 86, 98].

Persistent Diarrhea

Five case/control studies examined the association between *G. lamblia* and persistent diarrhea (≥14 days duration); 4 studies were carried out among children [104, 106, 107, 109] and one study enrolled adults [108]. Table 1 presents the pediatric studies that matched cases and controls according to potential confounders. The detection rate of *G. lamblia* was high in subjects with persistent diarrhea (9.8%–45%) and was 2.6- to 5.9-fold higher than in the control group [106, 107, 109].

Overview of the Longitudinal Studies

The salient features of the study designs and the results from 18 longitudinal studies undertaken in developing countries [24, 27, 30, 110–121] or populations in transition [29, 122, 123] are presented in Table 2. Some longitudinal cohort studies addressed the epidemiology and broad etiology of diarrheal disease [24, 110, 112, 113, 115–121], whereas others confined themselves to addressing the etiologic role of *G. lamblia* in association with diarrhea [27, 29, 30, 111, 114, 122, 123]. Children comprised the study target population except for 2 studies, one from Brazil [110] and the other from Egypt [112], that also included adult household members. The follow-up period in most studies was approximately 24 months [24, 29, 110, 112, 113, 116, 118, 120, 122, 123]. In the remaining studies follow-up was approximately 10–12 months [30, 114, 115, 117, 119], 3 years (average 1.5 years) [111], or 4 years (median 23 months) [27].

The analytical approach compared the prevalence of *G. lamblia* in stool samples that were obtained during diarrheal episodes with the prevalence of the parasite in stools obtained from asymptomatic children or in nondiarrheal stools that were obtained on a systematic predetermined basis (routine surveillance). Two studies compared the incidence of diarrhea in "*G. lamblia*–positive periods" with "*G. lamblia*–negative periods" [27, 29]. In 2 studies the incidence of diarrhea was compared between children who were positive for *G. lamblia* and children whose stool was negative for *G. lamblia* [30, 121]. Measurements of association were reported in only a fraction of the studies [27, 29, 30, 120, 121, 123]. Age- or multivariable-adjusted results were presented in 7 studies [27, 29, 30, 115, 117, 120, 121], whereas the rest presented unadjusted data [24, 111–113, 116, 122, 123]. In some studies, children with diarrhea who provided stool samples were matched with asymptomatic children who delivered stools during routine surveillance, for comparison [112, 113, 115, 117].

Many of the studies did not present the duration of diarrhea. Some studies presented a pooled analysis of acute and persistent diarrhea and a few presented separate analyses for acute vs persistent diarrhea (Table 2).

Only 3 of the 18 cohort studies showed a significantly increased risk of diarrhea in subjects infected with *G. lamblia* [24, 111, 114], while 7 studies showed no significant association between *Giardia* and diarrhea [27, 110, 113, 116–118, 121] (Table 2). One study investigated the length of carriage of *Giardia* in relation to the occurrence of diarrhea but found no significant association [122]. Interestingly, 7 cohort studies actually showed a lower risk for diarrhea in relation to the presence of *G. lamblia* in stools [29, 30, 112, 115, 119, 120, 123].

Is the Association Between G. lamblia and Diarrhea Age-Dependent?

We hypothesized that the association between *G. lamblia* and acute pediatric diarrhea among children in developing countries might be age-dependent; that is, the first infections that occur early in life might be associated with clinical diarrhea, whereas *Giardia* infections in older children might be largely asymptomatic (or may even lower the risk of acute diarrhea).

To address this hypothesis in this review, we abstracted data from studies that presented age-stratified results of the association between *G. lamblia* and diarrhea [24, 27–29, 70, 80, 118, 123]. An impediment to successful pursuit of this analysis was the heterogeneity of the age strata used for reporting data in

Table 1. Case/Control Studies on the Association Between *Giardia lamblia* and Diarrhea Among Children in Nonindustrialized Settings[a]

Study & Country	Study Period	Age	Definition of Diarrhea	*Giardia* Detection	No. Cases Sampling Frame	No. Controls Sampling Frame	*G. lamblia*–Positive Cases, %	*G. lamblia*–Positive Controls, %	OR (95% CI)	Matching/ Adjusting
Acute Diarrhea										
Orlandi [90] Brazil	2000–02	<6 y, 84.5% ≤2 y	Acute diarrhea: ≥3 loose stools in 24 h lasting ≥48 h	Microscopy (cysts)	470 ER	407 ER	1.27%	0.98%	1.30 (.31–6.32)	Age, sex, SES
Huilan [82] Multicenter study in Mexico, Pakistan, China, Myanmar, India	1982–85	<3 y, 47%-75% <1 y	Acute diarrhea: an increase in the number or volume of stools that lasted for ≤72 h. Children with a history of blood or mucus in stools & a temperature of ≥38°C also included	Microscopy (trophozoites or cysts)	Total 3640 outpatient	3279 community	3%	3%	1.00 (.70–1.45)	Region, age, sex, SES, ethnicity
Chatterjee [72] India	1982–83	0–14 y, 32.2% <1 y, 37.5% 1–4 y	Acute diarrhea	Microscopy (trophozoites or cysts)	152 hospital	272 health centers	2.6%	Urban: 25.6% Rural: 15%	0.10 (.04–.28) 0.15 (.04–.49)	Age
Mubashir [86] Pakistan	1983–85	<3 y, 73.6% 1–12 mo	Acute diarrhea of <72 h	Microscopy	402 hospital	402 hospital	2%	8.2%	0.23 (.10–.48)	Age, sex, SES, geographic region, ethnicity
Albert [68] Bangladesh	1994	0–5 y, 80% ≤2 y	Acute diarrhea ≥3 stools/ day	Microscopy	814 ICDDR,B	814 community	0.8%	2.9%	0.30 (.12–.68)	Age, neighborhood
Haque [80] Bangladesh[b]	2004–06	All ages: cases 30% 0–12 mo, controls 19% 0–12 mo	Acute diarrhea: ≥3 abnormal stools in 24 h. Dysentery: the presence of red blood cells, macrophages, or pus cells	EIA	1760 ICDDR,B	1145 clinic	4.5%	15.6%	0.26 (.19–.34)	Age, sex, SES
Hoge [103] Nepal	1994	0.5–5 y, mean age cases 19 mo	Acute diarrhea >3 unformed stools/24 h	Microscopy	124 outpatient	103 community	13%	18%	0.65 (.31–1.36)	Age, sex, neighborhood
Echeverria [73] Thailand	1985–86	<5 y, 80% <2 y	Acute diarrhea: ≥3 loose stools in the previous 24 h for <72 h	Microscopy	1230 outpatient	1230 outpatient	2%	1.3%	1.57 (.84–3.02)	Age
Bodhidatta [70] Thailand	2001–02	3 mo to 5 y, 75% <2 y	Admission due to acute diarrhea	EIA	207 hospital	227 hospital	15%	23%	0.58 (.35–.94)	Age
Loening [28] South Africa	1985–86	<6 y, 83% ≤2 y	≥5 stools/day for >1 d & <7 d	Microscopy (trophozoites or cysts)	373 outpatient	371 outpatient	6.4%	5.9%	1.09 (.60–2.00)	Age, clinic

Table 1 continued.

Study & Country	Study Period	Age	Definition of Diarrhea	*Giardia* Detection	No. Cases Sampling Frame	No. Controls Sampling Frame	*G. lamblia*–Positive Cases, %	*G. lamblia*–Positive Controls, %	OR (95% CI)	Matching/ Adjusting
Gascon [76] Tanzania[c]	1997	0–5 y, mean age: cases 1.9 y, controls 1.6 y	Acute diarrhea ≥3 watery/loose stools/24 h	Microscopy (trophozoites or cysts) Trophozoites	103 clinic	206 clinic	14.5%	15.5%	1.06 (.51–2.19) 1.82 (.76–4.34)	Age, sex, no. of alive siblings, distance to water source, & having a latrine at home
Meng [98] Cambodia[c]	2004–06	3 mo to 5 y, mean age: cases 11.4 mo, controls 31.2 mo	Acute diarrhea ≥3 watery/loose stools/24 h with ≥1 other enteric symptom	EIA	569 inpatient & outpatient	568 inpatient & outpatient	8.3%	21.7%	0.63 (.40–.99)	Age, sex, season
Persistent Diarrhea										
Sullivan [107] Gambia[d]	NA	0.5–3 y	>3 loose stools/day persisting for >2 wk	Microscopy	31 outpatient	33 healthy children outpatient	45%	12%	5.97 (1.50–28.20)	Age, sex
Bhandari [106] India[e]	NA	0–36 mo	Persistent diarrhea ≥3 liquid stools in 24 h lasting ≥14 d; acute diarrhea (<14 d).	Microscopy	175 household surveillance	175 healthy children; 175 acute diarrhea patients	20%	4.6% in each group	5.22 (2.40–12.32)	Age, nutritional status
Mukhopadhyay [109] Nepal[f]	1998–2004	<5 y	Persistent diarrhea: ≥3 liquid stools in 24 h lasting ≥14 d; acute diarrhea (<14 d).	Microscopy	253 inpatient, outpatient	100 healthy community controls, 100 acute diarrhea controls	Trophozoites: 9.8% Cysts: 14.2%	Trophozoites: healthy controls 2%; acute diarrhea 0% Cysts: healthy controls 6%; acute diarrhea 4%	Trophozoites: 5.37 (1.29–47.5) Cysts: 2.60 (1.03–7.79)	Nutritional status

Abbreviations: CI, confidence interval; EIA, enzyme immunoassay; ER, emergency room; ICDDR,B, International Centre for Diarrhoeal Disease Research, Bangladesh; OR, odds ratio; SES, socioeconomic status.

[a] The ORs and 95% CIs were calculated using the raw data that were presented in the original manuscripts, except for 2 studies that presented adjusted OR: Gascon et al [76] and Meng et al [98].

[b] From the study of Haque et al [80] we abstracted data only on children ≤5 years of age.

[c] The adjusted ORs that appeared in the manuscript are presented.

[d] Cases were children with chronic diarrhea and malnutrition; data on the healthy control children are presented.

[e] The results are similar when the control group was the healthy children or the patients with acute diarrhea.

[f] OR was calculated while including the healthy control children.

Table 2. Cohort Studies That Addressed the Role of *Giardia lamblia* in Diarrhea

Study & Country	Study Population	Definition of Diarrhea	Surveillance of Diarrhea	Detection of *Giardia*	No. Diarrheal Stools	No. Nondiarrheal Stools	*G. lamblia*–Positive Diarrhea Stools, %	*G. lamblia*–Positive Nondiarrhea Stools, %	OR/RR (95% CI)[a]
Guerrant [110] Brazil	297, household members, all ages	A significant change in bowel habits: decreased consistency or increased frequency. The duration of diarrhea was presented for other pathogens than *Giardia*.	Daily surveillance was conducted by the mother, and by weekly home visits performed by research assistants.	Microscopy	150	32	6.7%	12.5%	0.50 (.13–2.35)
Schorling [113] Brazil[b]	175, age <5 y	An increase in stool frequency or decrease in consistency, which lasted ≥1 d and was separated from another episode by 3 diarrhea-free days. Acute diarrhea <14 d. Persistent diarrhea ≥14 d,	Home visits 3 times/wk	Microscopy	Acute diarrhea: 50, persistent diarrhea, 40	38	Acute diarrhea: 22%, persistent diarrhea:17.5%	13.2%	1.86 (.59–6.45) 1.40 (.39–5.26)
Newman [111] Brazil	157 newborns followed up from birth	≥3 unformed stools in 24 h. Acute diarrhea lasting <14 d. Persistent diarrhea ≥14 d.	Home visits 3 times/wk.	Microscopy	Acute diarrhea: 514, persistent diarrhea: 97	299	Acute diarrhea: 7.6%, persistent diarrhea: 20.6%	7.4%	1.03 (.58–1.84) 3.27 (1.62–6.62)
Black [116] Peru	153 newborns followed from birth	≥1 d with liquid stools totaling 6 for infants <1 mo, 5 for infants aged 1 mo & 4 for older infants. New episode began after 2 free-illness days. The duration of diarrhea was presented for other pathogens than *Giardia*	Thrice-weekly home visits	Microscopy	952	1973	0.7%	0.8%	0.91 (.35–2.18)
Kaminsky [119] Honduras	266, 101 controls, Age <6 y	An increase in the usual number & change in the consistency of stools for ≥1 d. Acute & persistent diarrhea	Twice-weekly visits	Microscopy (troph. or cysts)	848	101	29%	57%	0.50 (.42–.63)
Hollm-Delgado [27] Peru[c]	220 infants followed up from birth to age 35 mo	≥3 liquid/semi liquid stools/d in 2 consecutive days. The duration of diarrhea was not presented.	Daily home visits	Microscopy	3911	16 973	6%	6.2%	0.95 (.79–1.13)
Boeke [121] Colombia[d]	442, age 5–12 y	Maternal reports. The outcome was diarrhea days.	Daily reports in pictorial diaries	Microscopy (cysts)	Positive children: 28	Negative children: 414	4.0	4.7	0.73 (.52–1.02)
Stanton [24] Bangladesh	343, age <6 y	≥ 3 loose stools in 24 h; new episode began after 14 d without diarrhea. Duration of diarrhea was not presented.	Fortnightly maternal interviews	Microscopy (cysts)	225	1006	11%	4%	2.61 (1.53–4.37)
Baqui [117] Bangladesh	705, age <5 y	≥3 liquid/loose or watery stools or at least 1 bloody stool in 24-hours, Acute diarrhea <14 d, persistent diarrhea ≥4 d	Home visits every fourth day.	Microscopy	Acute diarrhea: 161 Persistent diarrhea: 167	165	Acute diarrhea: 0.6% Persistent diarrhea: 1.2%	1.8%	0.33 0.68
Hasan [118] Bangladesh	252 newborns followed from birth for 2 y	≥3 liquid stools in 24 h or any loose stools accompanied with blood in 24 h. Acute diarrhea <2 wk. Persistent diarrhea ≥2 wk. Data for *Giardia* were presented in a pooled analysis of acute & persistent diarrhea.	Twice-weekly home visits	Microscopy (troph.)	1748	5679	13.2%	13%	1.01 (.86–1.19)
Zaki [112] Egypt	2563, household members, all ages	Reports of the family speak person. The duration of diarrhea was not presented	Twice-weekly home visits	Microscopy	3080	703	44.3%	56.0%	0.63 (.53–.74)
Fraser [123] Israel	164 Bedouin newborns followed from birth to age 23 mo	≥3 soft stools in 24 hours. For infants aged <1 mo ≥4 soft stools. The duration of diarrhea was not presented.	Through the local clinics and hospital, and through monthly and weekly maternal interviews.	Microscopy (cysts)	239	730	22.3%	28.5%	0.8 (.7–.9)

Table 2 continued.

Study & Country	Study Population	Definition of Diarrhea	Surveillance of Diarrhea	Detection of *Giardia*	No. Diarrheal Stools	No. Nondiarrheal Stools	*G. lamblia*–Positive Diarrhea Stools, %	*G. lamblia*–Positive Nondiarrhea Stools, %	OR/RR (95% CI)[a]
Bilenko [29] Israel[e]	238 Bedouin newborns followed from birth to age 23 mo	Maternal reports. The duration of diarrhea (acute vs persistent was not presented).	Weekly maternal interviews	EIA	349	8591	16%	23%	0.65 (.47–.91)
Bilenko [29] Israel[f]	238 Bedouin newborns followed from birth to age 23 mo	Maternal reports. The duration of diarrhea (acute vs persistent was not presented).	Weekly maternal interviews	EIA	1453 *Giardia*-positive months	3001 *Giardia*-negative months	6.7%	6.7%	1.09 (.81–1.46)
Molbak [115] Guinea-Bissau	471–755 children	Maternal reports. Data for *Giardia* were presented in a pooled analysis of acute and persistent diarrhea.	Weekly visits	Microscopy (troph. or cysts) Troph.	1219	511	19.1%, 9.3%	25%, 9.8%	0.8 (.6–1.0) 1.1 (.7–1.5)
Chunge [114] Kenya[g]	84 children aged 10–28 mo	Maternal report. The duration of diarrhea was not presented.	Weekly surveillance	Microscopy (troph. or cysts)	1227	537	78.8%	68.6%	1.69 (1.15–2.54)
Veenemans [30] Tanzania[h]	558, age 6–60 mo	Diarrhea: any report by the caretaker or ≥3 stools in 24 h. The duration of diarrhea was not presented.	Health-facility based surveillance	EIA	Positive children: 192	Negative children: 336	Overall: 0.43 Micro-nutrients: 0.58 No micro-nutrients: 0.29	0.68 0.63 0.72	0.84 (.64–1.09) 1.04 (.75–1.43) 0.56 (.34–.90)
Valentiner-Branth [120] Guinea-Bissau[i]	200 newborns followed from birth to age 2 y	Maternal report. The duration of episode was not presented.	Weekly home visits	Microscopy	na	na	na	na	0.64 (.46–.89)

Abbreviations: CI, confidence interval; EIA, enzyme immunoassay; na, not available; OR, odds ratio; RR, rate ratio; Troph, trophozoites.

[a] ORs and CIs were calculated using the raw data presented in the original manuscripts for studies that did not provide measurement of association [24, 110–114, 116, 118, 119]. The measurement of association was provided in the study of Baqui et al [117] (OR) Molbak et al [115] (multivariable adjusted OR), Fraser et al [123] (OR), Bilenko et al [29] (age-adjusted Mantel-Haenszel OR), Hollm-Delgado et al [27] (multivariable adjusted RR), Boeke et al [121] (multivariable adjusted incidence RR), Veenemans et al [30] (adjusted hazard ratio), Valentiner-Branth et al [120] (multivariable adjusted OR).

[b] OR was calculated for acute diarrhea and for persistent diarrhea separately, whereas the comparison group was nondiarrhea.

[c] Adjusted RR for the incidence of diarrheal episodes in *G. lamblia*–positive weeks as compared with *G. lamblia*–negative weeks.

[d] In the study of Boeke et al [121], the incidence of diarrhea days was calculated by dividing the total number of diarrhea days by child years of observation in children who were positive and negative for *Giardia*.

[e] This study [29] presented 2 analyses; this analysis reflects the detection rates of *G. lamblia* in diarrhea stools compared with nondiarrheal stools. Please see the second analysis in the next row.

[f] This study [29] presented 2 analyses; this analysis reflects the adjusted RR for the incidence of diarrheal episodes in *G. lamblia*–positive months as compared with *G. lamblia*–negative months. The first analysis is presented in the previous row.

[g] In the study of Chunge et al [114], the results reflect the detection of *G. lamblia* in stools in relation to maternal reports on diarrhea.

[h] In the study of Veenemans et al [30], the incidence of diarrheal episodes was calculated as the number of episodes divided by child-years of follow-up in children who tested positive and negative for *G. lamblia* at baseline. The results reported in this table are for any reported diarrhea.

[i] Valentiner-Branth et al [120], reported the odds ratio of maternal report on diarrhea during weekly home visits in which stool samples were collected if the child had or did not have diarrhea. The OR in this study reflect the odds of diarrhea during infection with *Giardia*.

Table 3. Association Between *Giardia lamblia* Infection and Diarrhea by Age Groups

Study & Country	Age Groups (mo)	No. *Giardia* Positive/No. Diarrhea	No. *Giardia* Positive/No. Controls	OR (95% CI)[a]
Loening [28] South Africa	0–6	1.2% (n = 80)	1.7% (n = 58)	0.72 (.02–27.05)
	7–12	7.1% (n = 113)	4.3% (n = 115)	1.68 (.52–5.78)
	13–24	8.5% (n = 130)	6.5% (n = 124)	1.34 (.51–3.60)
	25–72	8% (n = 50)	10.8% (n = 74)	0.72 (.18–2.53)
Fraser [123] Israel	≤3	4.2%	1.1%	4.1 (1.1–15.3)
	4–6	5.2%	3.2%	1.6 (.6–4.2)
	7–9	8.7%	11.1%	0.8 (.4–1.4)
	10–12	13.4%	23%	0.5 (.3–0.8)
	13–15	31.8%	33.8%	0.9 (.6–1.3)
	16–18	27.8%	35.9%	0.7 (.4–1.1)
	19–21	41.4%	37%	1.2 (.8–1.9)
	22–24	37.9%	36.1%	1.0 (.6–1.9)
Stanton [24] Bangladesh	<12	3/38 (8%)	0/131 (0%)	…
	12–23	4/55 (7%)	12/173 (7%)	1.05 (.28–3.30)
	24–72	17/132 (13%)	32/702 (5%)	3.10 (1.63–5.72)
Hasan [118] Bangladesh	0–5	2.7% (n = 300)	2.6% (n = 1429)	1.03 (.45–2.16)
	6–11	9.2% (n = 532)	7.3% (n = 1382)	1.29 (.89–1.83)
	12–17	16.5% (n = 520)	16.9% (n = 1405)	0.98 (.74–1.28)
	18–23	22.2% (n = 396)	25.1% (n = 1463)	0.85 (.65–1.11)
Haque [80] Bangladesh	0–12 mo	38/1088 (3.5%)	18/485 (3.7%)	0.94 (.53–1.70)
	1–5 y	4/672 (6.1%)	160/660 (24.2%)	0.20 (.14–0.29)
	6–14 y	31/279 (11.1%)	146/457 (31.9%)	0.27 (.17–0.40)
	15–40 y	91/1222 (7.4%)	92/753 (12.2%)	0.58 (.43–0.79)
	>40 y	4/385 (1%)	24/220 (10.9%)	0.09 (.03–0.24)
Bodhidatta [70] Thailand	3–12	5/85 (6%)	10/103 (10%)	0.58 (.17–1.77)
	13–24	12/79 (15%)	28/77 (36%)	0.31 (.14–0.68)
	25–59	14/43 (33%)	15/47 (32%)	1.00 (.42–2.53)
Studies on the incidence of diarrhea in *Giardia*-positive and -negative periods				

Table 3 continued.

Study & Country	Age Groups (mo)	No. *Giardia* Positive/No. Diarrhea	No. *Giardia* Positive/No. Controls	OR (95% CI)[a]
		No. diarrheal episodes/*Giardia*-positive periods	No. diarrheal episodes/ *Giardia*-negative periods	OR/RR (95% CI)
Bilenko [29] Israel[b]	0–6	3/99	100/1565	0.46 (.11–1.53)
	7–12	45/508	80/914	1.01 (.68–1.51)
	13–18	50/846	20/522	1.58 (.90–2.78)
Hollm-Delgado [27] Peru[c]	0–5	10/188	157/4404	1.56 (.7–3.3)
	6–11	25/402	319/4321	0.86 (.6–1.2)
	12–17	70/762	298/3291	1.02 (.8–1.4)
	18–23	49/901	146/2289	1.00 (.7–1.4)
	24–35	81/1658	135/2668	0.94 (.7–1.4)

Abbreviations: CI, confidence interval; OR, odds ratio; RR, rate ratio.

[a] We calculated the ORs and 95% CIs for Bodhidatta et al [70], Hasan et al [118], Stanton et al [24], and Haque et al [80].

[b] Bilenko et al [29] presented data on the number of diarrheal episodes in months in which *G. lamblia* was detected compared with months in which *G. lamblia* was not detected, and presented OR.

[c] Hollm-Delgado et al [27] presented data on diarrheal stools that were positive for *Giardia* among all *Giardia*-positive stools, compared with diarrheal stools that were negative for *Giardia* among all *Giardia*-negative stools, and presented the adjusted RR.

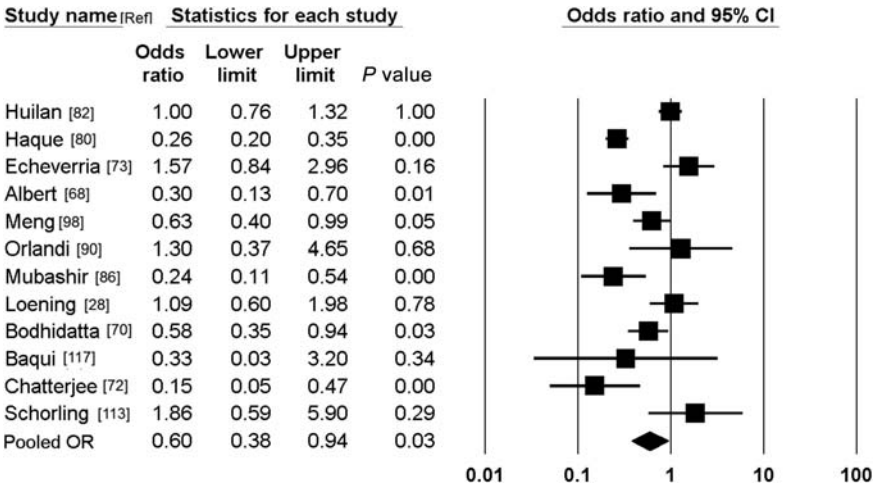

Figure 1. Forest plot of studies on the association between *Giardia lamblia* infection and acute diarrhea among children from developing countries. The odds ratio (OR) and 95% confidence interval (CI) of each study included in the meta-analysis and the pooled OR and 95% CI obtained using the random effects model are presented. Squares and bars represent individual study OR and 95% CI. Diamond represents pooled OR and 95% CI.

the different studies (Table 3). Among these, one study reported a significant increased risk for diarrhea among *Giardia*-infected subjects in the youngest age group (<3 months of age) and a lower risk or no association between *Giardia* and diarrhea in older ages [123] (Table 3).

META-ANALYSIS OF *G. LAMBLIA* AND ENDEMIC PEDIATRIC DIARRHEA

The Association Between *G. lamblia* and Acute Diarrhea

Ten case/control studies [28, 68, 70, 72, 73, 80, 82, 86, 90, 98] and 2 cohort studies [113, 117] that enrolled children from developing countries or other nonindustrialized settings were included in the meta-analysis because their design and execution revealed no fundamental flaws (as explained in the Methods section). From the study of Haque et al [80], we abstracted data only on children aged ≤5 years. These 12 studies [28, 68, 70, 72, 73, 80, 82, 86, 90, 98, 113, 117] fulfilled the inclusion criteria of presenting the outcome variable of acute diarrhea, they matched or controlled for potential confounders, and the study lasted at least 1 year. Using the random effects models, the pooled OR was 0.60 (95% CI, .38–.94; $P = .03$) (Figure 1). This suggests that the presence of *Giardia* infection actually diminished the likelihood of having acute diarrhea among children from developing countries. The heterogeneity test was statistically significant; χ^2 77.9 ($P < .001$), I^2 85.9%.

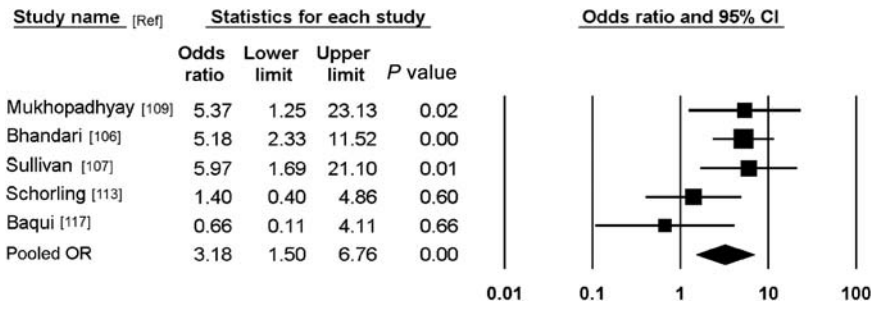

Figure 2. Forest plot of studies on the association between *Giardia lamblia* infection and persistent diarrhea among children from developing countries. The odds ratio (OR) and 95% confidence interval (CI) of each study included in the meta-analysis and the pooled OR and 95% CI obtained using the random effects model are presented. Squares and bars represent individual study OR and 95% CI. Diamond represents pooled OR and 95% CI.

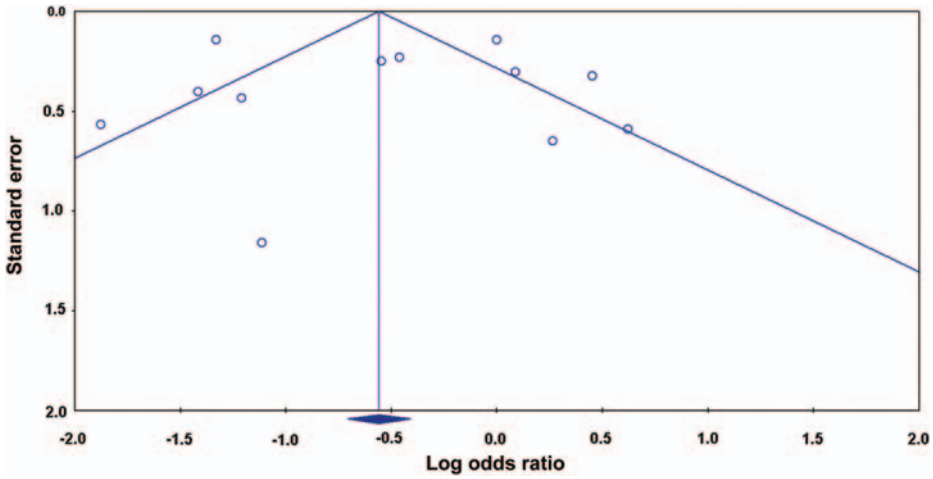

Figure 3. Funnel plot of studies included in the meta-analysis on the association between *Giardia lamblia* infection and acute diarrhea. The log odds ratio (OR) of each study on the x-axis is plotted against the corresponding standard error on the y-axis. The studies are represented in the funnel plot as opened circles. The rhombus shape at the x-axis reflects the log of the pooled OR obtained by using the random effects model.

The Association Between *G. lamblia* and Persistent Diarrhea

Two cohort studies [113, 117] and 3 case/control studies [106, 107, 109] that fulfilled the inclusion criterion of matching between cases and controls and that lasted ≥1 year presented data on persistent diarrhea as an outcome. Results from these 5 studies were combined using the random effects model. The pooled OR was 3.18 (95% CI, 1.50–6.76; $P < .001$), suggesting that *G. lamblia* infection significantly increases the likelihood of persistent diarrhea (Figure 2). The heterogeneity χ^2 test was 7.22 ($P = .125$), I^2 44.6%.

Assessing the Potential of Publication Bias

Figure 3, which presents funnel plots of studies included in the meta-analysis on acute diarrhea, appears visually symmetrical. The Egger regression intercept of the meta-analysis on acute diarrhea studies was 0.32 (95% CI, −3.18 to 3.83; 2-tailed $P = .83$). These results provide no hint of publication bias. Figure 4 shows the cumulative meta-analysis of studies on acute diarrhea. There is no evidence that the addition of the small studies affected the direction of the association between *G. lamblia* infection and the likelihood of acute

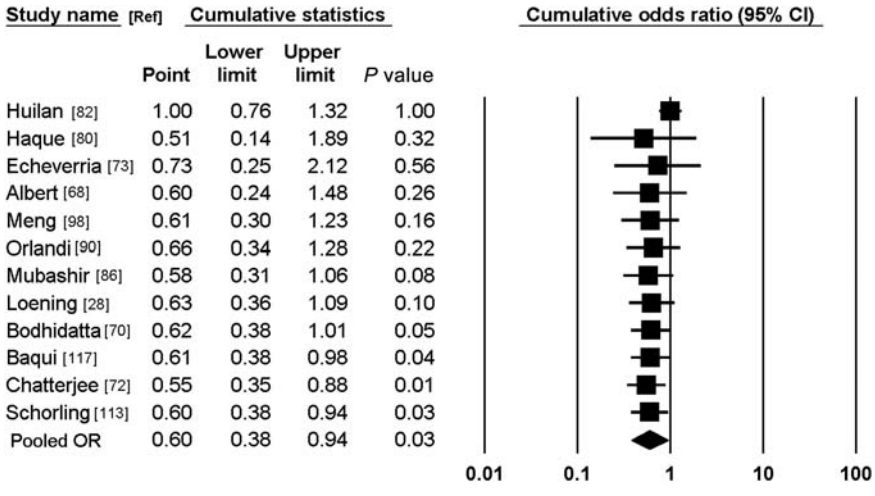

Figure 4. Cumulative meta-analysis of the association between *Giardia lamblia* and acute diarrhea among children from developing countries by study sample size. The change in the pooled odds ratio (OR) is described by adding studies according to their sample size, starting with the largest study. Squares and bars represent individual study OR and 95% CI. Diamond represents pooled OR and 95% CI.

diarrhea in children from developing countries. The Egger regression intercept of the meta-analysis of studies on persistent diarrhea was –2.57 (95% CI, −8.93 to 3.79; 2-tailed $P = .28$).

G. LAMBLIA AND TRAVELERS' DIARRHEA

Early reports of the etiology of travelers' diarrhea [124–126] emphasized enterotoxigenic *Escherichia coli* as the most frequent pathogen, present in approximately 30% of cases [124]. A recent review showed *G. lamblia* in 1.3% and 1.6% of travelers with diarrhea whose destination was Latin America or Africa, respectively, in comparison with 6.2% and 5.7% of travelers to South and Southeast Asia [124]. Only a few studies investigated in parallel the presence of *Giardia* in stools of travelers who did not develop diarrhea (as controls). Our review considered only studies that included a control group of travelers without diarrhea. We identified 12 case/control [127–138] and 2 cohort studies [139, 140] that examined the role of *Giardia* in travelers' diarrhea. Characteristics of these studies are shown in Table 4. *Giardia lamblia* was detected by means of stool microscopy [128, 132–140], except for one study that used enzyme immunoassay [131] and 2 studies that did not specify their method for detecting *G. lamblia* [129, 130].

Studies from the 1970s showed a significant association between travelers' diarrhea and *Giardia* infection among travelers to Leningrad and other sites in the former Soviet Union [133, 139]. Studies of travelers to Nepal revealed that *G. lamblia* was significantly and strongly associated with diarrhea [128, 131], whereas among travelers to Mexico no significant association was found between *G. lamblia* and diarrhea [132, 134, 140]. Three other studies of travelers from Canada, Spain, and the Netherlands (whose destinations were Africa, Asia, and Latin America) also showed a significant positive association between *G. lamblia* and diarrhea or gastrointestinal symptoms [129, 137, 138], and in particular with prolonged (>7 days) and persistent diarrhea (>14 days) [131, 138] (Table 4).

G. LAMBLIA GENOTYPES AND CLINICAL ILLNESS

Experimental challenge studies with *G. lamblia* in which healthy volunteers were inoculated enterally with trophozoites of 2 distinct human isolates of *G. lamblia*, designated GS/M and Isr, demonstrated the establishment of infection and elicitation of clinical illness only among participants who were challenged with the GS/M isolate [14], belonging to *Giardia* assemblage B. This study established the concept that there exists variability among *Giardia* strains with respect to their pathogenicity for humans [14]. Animal experiments support this concept of variable pathogenicity among *Giardia* strains [141].

A few recent studies that were undertaken following the availability of techniques to genotype *G. lamblia* have suggested a possible association between *G. lamblia* genotypes A or B and clinical illness [63, 80, 105, 142–148] (Table 5). Nevertheless, one must exercise caution in drawing conclusions from these preliminary reports as the sample size of each of these studies was small (6–138 diarrheal cases infected with *G. lamblia* and 6–199 nondiarrhea subjects infected with *G. lamblia*). These studies also varied by design, study population, and outcome under investigation (Table 5).

The correlation between *G. lamblia* genotypes and severity of diarrheal illness was examined in an industrialized-country setting among 18 Dutch patients aged 8–60 years with diarrhea and *G. lamblia* infection who visited their general practitioner [149]. Assemblage A *Giardia* was found exclusively among the patients with intermittent/mild disease, while all assemblage B *Giardia* was detected among the patients with more severe cases of diarrhea [149]. Larger studies on the relationship between *G. lamblia* genotype and diarrhea or other gastrointestinal symptoms mostly showed that genotype B was more common (70%–96% in the controls) than genotype A [80, 105, 143, 145, 146, 148, 150], but a higher detection rate of genotype A was found among the symptomatic patients than the controls. A significant association between genotype A and increased risk of diarrhea or other gastrointestinal symptoms was reported from case/control and longitudinal studies [80, 105, 142, 143, 148]. The relationship between *G. lamblia* assemblages and diarrhea was examined in a reanalysis [150] of data from a longitudinal study. This study showed no significant difference between *G. lamblia* genotypes and the number of diarrheal episodes; 0.89 (±0.6) in assemblage A, 1.3 (±1.5) in assemblage B, and 0.80 (±0.84) in mixed infections ($P = .58$) [150]. A study from Sweden compared the distribution of diarrhea and other gastrointestinal symptoms in patients infected with assemblage A *Giardia* (n = 51) and subjects infected with assemblage B (n = 87). The reports on diarrhea were similar between the 2 groups (94% and 99% in assemblage A and B, respectively) but flatulence was more common in subjects infected with assemblage B (85%) than A (65%) [151].

DISCUSSION

The confusing, often conflicting, information in the literature on the role of *G. lamblia* as an enteric pathogen capable of causing diarrheal illness among young children in developing countries led us to undertake this systematic review. Four fundamental conclusions can be drawn from this exercise: (1) *G. lamblia* is capable of causing both acute and persistent

Table 4. Studies That Addressed the Role of Giardia in Travelers' Diarrhea

Study	Country of Origin	Definition of Outcome	Sampling	Age	Travel Destination	*Giardia*-Positive Cases/Total Cases (%)	*Giardia*-Positive Controls/Total Controls (%)	RR/OR (95% CI)[a]	Matching/ Adjusting
Andersson [139]	Sweden	Gastrointestinal symptoms	Students who traveled to Leningrad	Adult students	Leningrad	27/27 (100%)	3/11 (27.3%)	3.66 (1.75–10.26)	None
Brodsky [133]	US	Gastrointestinal symptoms	Tourists, CDC surveillance	All ages	Former Soviet Union	83/153 (54.3%)	8/153 (5.2%)	21.49 (10.11–44.49)	None
Merson [140]	US, Canada, Netherlands, England	The occurrence between 12 h after arrival to Mexico City and 5 d after departure of any unformed stool not attributed to a preexisting condition plus ≥1 enteric symptom. Or ≥3 watery stools in 24 h	Physicians & their family members	Mainly adults	Mexico	1/51 (2%)	1/43 (2.3%)	0.84 (.01–67.49)	None
DuPont [132]	US, Venezuela, Mexico	Acute diarrhea: unformed bowel movements at a daily rate twice of the usual rate plus ≥1 enteric symptom	University clinic	Adult students	Mexico	US 6% (total cases 77), LA 18% (total cases 18)	US 3% (total controls 67), LA 11% (total controls 27)	2.26 (.43–17.20), 1.60 (.19–13.43)	Country of origin, length of stay in Mexico
Bolivar [134]	US, Venezuela, Mexico	Unformed bowel movement at daily rate twice that of the subject's usual rate & ≥1 other enteric symptom	University clinic	Adult students	Mexico	3/91 (3.3%)	2/74 (2.7%)	1.23 (.18–10.54)	Country of origin, length of stay in Mexico
Back [127]	Sweden	≥2 abnormal loose stools/d	Swedish battalion in United Nations forces	Adults	Cyprus	1/79 (1.3%)	0/66 (0%)		Serving conditions (next bedfellow)
Echeverria [135]	US	≥3 loose stools or ≥2 loose stools with other enteric symptom	Soldiers who attended a clinic	Adults	Philippines	3/152 (2%)	2/58 (3.5%)	0.56 (.06–6.94)	None
Hoge[b] [136]	Foreign residents & tourists	Change in normal bowel movements with ≥3 loose stools in 24 h	CIWEC, USEM	All ages	Nepal	7/148 (4.7%)	1/95 (1%)	4.67 (.58–212.52)	Group matching by clinic & season
Shlim [128]	Tourists, expatriates	Change in normal bowel movements & ≥3 loose stools in 24 h	CIWEC	≥18 y	Nepal	25/189 (13.2%)	3/112 (2.6%)	5.54 (1.62–29.23)	None
Gascon [137]	Spain	Diarrhea that occurred between 12 h after arriving in & 5 d after departing from the travel country. Diarrhea ≥3 watery stools in 24 h, or unformed stools plus enteric symptom	Tropical Medicine Department	NA	Asia, Africa, Central & Latin America	11/165 (6.7%)	3/165 (1.8%)	3.86 (.99–21.86)	Area visited, controls were relatives or travel companions of cases

Table 4 continued.

Study	Country of Origin	Definition of Outcome	Sampling	Age	Travel Destination	*Giardia*-Positive Cases/Total Cases (%)	*Giardia*-Positive Controls/Total Controls (%)	RR/OR (95% CI)[a]	Matching/ Adjusting
Schultsz [138]	Netherlands	≥3 loose stools in 24 h, any number of watery stools in 24 h, or 1–2 loose stools in 24 h plus ≥1 enteric symptom	Outpatient Department for Tropical Diseases	2–75 y	Asia, Africa, Central & Latin America	Acute 2% (total cases 49), persistent 16.4% (total cases 116)	4.9% (total controls 102)	0.40 (.01–3.78), 3.80 (1.30–13.48)	None
Boggild [129]	Canada	Diagnosis of giardiasis	Tropical Disease Unit. (GeoSentinel Network)	Mean 37.3 y	International travel	69/1622 (4.3%)	5/1906 (0.3%)	16.9 (6.8–41.9)	None
Paschke [130]	Germany	≥3 unformed stools in 24 h plus ≥1 symptom of enteric infection	Department of Infectious Diseases & Tropical Medicine.	2–80 y	Asia, Latin America, Europe, other	7/114 (6.1%)	3/56 (5.4%)	1.16 (.25–7.20)	None
Pandey [131]	US, Japan, Australia, New Zealand, Western Europe	≥3 unformed stools in 24 h	CIWEC	>18 y	Nepal	Overall 42/372 (11.3%) ≤7 d 7%, >7 d 26%	5 (2.9%)	3.75 (1.40–9.98), 2.48 (.95–7.52), 11.78 (4.41–35.90)	Age, sex, nationality, resident/ tourist status, length of stay in Nepal, season

Abbreviations: CDC, Centers for Disease Control and Prevention; CI, confidence interval; LA, Latin American (Venezuela and Mexico); CIWEC, Canadian International Water and Energy Consultants; OR, odds ratio; RR, rate ratio; USEM, US Embassy Medical Care.

[a] ORs and 95% CIs were calculated using the abstracted data from each study. For the study of Andersson et al [139], RR was calculated. Boggild et al [129] reported crude OR and Pandey et al [131] reported adjusted OR.

[b] Data from the study of Hoge et al [136] were abstracted on 148 cases of diarrhea among which coccidian-like organisms were not identified. Cases and controls from both clinics (CIWEC and USEM) were pooled.

Table 5. Association Between *Giardia lamblia* Assemblage and Diarrhea or Other Gastrointestinal Symptoms

Study & Country	Design	Subjects	Outcome	No. Cases Genotyped	No. Controls Genotyped	Genotype A Cases, No. (%)	Genotype A Controls, No. (%)	Genotype B Cases, No. (%)	Genotype B Controls, No. (%)	OR (95% CI)[a]
Paintlia [147] India	Case series	Adults from gastroenterology & dermatology clinics	Gastrointestinal symptoms: diarrhea, weight loss, abdominal pain	6	6	4 (66.7%)	1 (16.7%)	2 (33.3%)	5 (83.3%)	10.0 (.43–588.32)
Eligio-Garcia [63] Mexico	Case series	6–12 y old children	Chronic/recurrent diarrhea & abdominal pain	6	7	6 (100%)	7 (100%)	0 (0%)	0 (0%)	
Al-Mohammed [144][b] Saudi Arabia	Cross-sectional	Primary school-age children 6–12 y	Acute & chronic diarrhea	24	16	7 (29.2%)	16 (100%)	15 (62.5%)	0 (0%)	
Molina [146][c] Argentina	Cross-sectional	2–14 y old enrolled at health centers or public schools	Symptoms: diarrhea, anorexia, vomiting, abdominal pain	50	41	8 (16%)	6 (14.6%)	42 (84%)	35 (85.4%)	1.11 (.30–4.28)
Aydin [143] Turkey	Case/control	Patients from Dept of Infectious Disease & Gastroenterology	Diarrhea	20	24	17 (85%)	2 (8%)	3 (15%)	22 (92%)	62.33 (9.13–480.26)
Sahagun [148][d] Spain	Case/control	Ages 2–72 y from outpatient clinic suspected of parasitosis	Symptoms: diarrhea, nausea, abdominal pain/cramps, weight loss, flatulence	55	49	29 (52.7%)	14 (28.5%)	26 (47.3%)	35 (71.5%)	2.79 (1.23–6.38)
Haque [105][e] Bangladesh	Matched case/control	All ages, cases from ICDDR,B, Hospital controls	Diarrhea	84	199	16 (19.5%)	20 (10.5%)	68 (80.5%)	179 (89.5%)	2.11 (1.04–4.26)
Haque [80][f] Bangladesh	Matched case/control	All ages, cases from ICDDR,B, Clinic controls	Acute diarrhea	138	184	29 (21%)	10 (5.4%)	109 (79%)	174 (94.6%)	4.63 (2.20–10.27)
Read [142] Australia	Longitudinal	Children in day care centers age <5 y	Diarrhea	9	14	6 (66.7%)	1 (7.1%)	3 (33.3%)	13 (92.9%)	26.0 (2.2–304.7)

Table 5 continued.

Study & Country	Design	Subjects	Outcome	No. Cases Genotyped	No. Controls Genotyped	Genotype A Cases, No. (%)	Genotype A Controls, No. (%)	Genotype B Cases, No. (%)	Genotype B Controls, No. (%)	OR (95% CI)[a]
Ajjampur [145][g] India	Longitudinal	Newborns followed till age 3 y	Acute & intermediate diarrhea (<14 d)	45	50	5 (11.1%)	2 (4%)	40 (89.9%)	48 (96%)	3.00 (.46–32.74)

Abbreviations: CI, confidence interval; ICDDR,B, International Centre for Diarrhoeal Disease Research, Bangladesh; OR, odds ratio.

[a] The OR presented here reflects the odds of *G. lamblia* genotype A infection among the cases in comparison to odds of genotype A infection in the control group. The calculations of OR (95% CI) were made using the raw data in the original manuscripts when the authors did not present the measurement of association [80, 143–145, 148].

[b] Two samples with mixed infections among the cases were not included in the calculation.

[c] Samples with mixed infections (n = 3) were not included in the calculation. Cases were children with gastrointestinal symptoms.

[d] Four samples had mixed A and B genotypes, 2 among the symptomatic and 2 among the asymptomatic patients [148]; they were not included in the data presented in this table. Among genotype A isolates, only subgenotype AII was identified [148].

[e] A total of 267 *G. lamblia*–positive stool specimens were genotyped, among which 16 samples harbored mixed A and B genotypes that were counted twice by the authors, once as A genotype and once as B genotype [105].

[f] *G. lamblia*–positive stools of 144 and 199 cases and controls were genotyped; of these 6 and 15 were mixed genotype A and B infections [80], and they were not included in the calculations presented in this table. Part of the *G. lamblia* genotypes included in this study was reported in an earlier report [105].

[g] Five mixed infections among the cases and 1 in the control group were excluded from the analysis.

diarrheal illness in adult and pediatric hosts who reside in industrialized countries, including following exposure when they travel to developing countries. (2) *G. lamblia* does not generally cause acute pediatric diarrhea among infants and children in developing countries, although limited data suggest that infants in the first trimester of life may experience acute clinical diarrhea in response to presumed initial *G. lamblia* infections. (3) *G. lamblia* is positively associated with persistent diarrhea among children in developing countries. (4) Genotyping suggests that 2 *G. lamblia* genotypes (assemblages A and B) may be particularly pathogenic for humans.

Among residents of industrialized countries, evidence from experimental challenge studies of adult volunteers [14, 15], investigations of (particularly water-borne) outbreaks of diarrheal disease [11, 16–18, 23], and investigations of travelers who visit developing countries or known endemic areas [128, 129, 133, 137] collectively and convincingly document that *G. lamblia* can cause acute diarrheal illness and other gastrointestinal disease. In contrast, as summarized in this review, contradictory results have been reported from epidemiological studies performed in subjects residing in developing countries [24, 72, 82].

Herein we provide the first systematic review and meta-analysis that attempts to address the etiologic role of *G. lamblia* in relation to diarrheal illness among children from developing countries or other nonindustrialized settings where *Giardia* is highly endemic. In systematically reviewing the literature, it became apparent that among the many published studies that explored a possible association between *G. lamblia* and diarrhea, few utilized rigorous design methodology and analytical techniques. For example, few studies controlled for potential confounders and many lacked the statistical power to detect differences between patients with diarrhea and controls without diarrhea. There were very few birth cohort studies, thus the age of first infection could not be assessed. Some studies did not differentiate between the clinical syndromes of acute vs persistent diarrhea, which is critical for analyzing data on *Giardia* infections; consequently, misclassification of the outcome variable may have ensued. Finally, some studies were limited in duration, covering <1 year.

Accordingly, we limited our analysis to the case/control and cohort studies that utilized rigorous methodology and controlled for potential confounders, lasted ≥ 1 year, and clearly defined the outcome variable (ie, acute vs persistent diarrhea). In so doing, we found there was no significant association between the presence of *Giardia* in stools and increased risk of acute diarrhea among children living developing countries or nonindustrialized settings [28, 73, 82, 90, 113, 117]. Indeed, there was evidence of a significant inverse association between the presence of *Giardia* in stools and acute diarrhea among children in developing country or other nonindustrialized

settings [68, 70, 72, 80, 86, 98]. A pooled analysis of the studies that utilized rigorous methodology showed that *G. lamblia* was associated with a 40% lower likelihood of acute diarrhea in children from developing countries ($P = .03$) (Figure 1).

One may invoke differences in the host, the parasite, or host-parasite interactions to explain the strikingly distinct responses to *Giardia* exposure among children and adults from industrialized countries vs developing countries. The former are at risk of developing acute diarrhea when they encounter *G. lamblia*, whereas pediatric subjects in the latter settings experience apparent innocuity or even a protective effect of *Giardia* against acute diarrhea when infected with this protozoan. One possible explanation may relate to the age of initial exposure and the frequency of subsequent reexposure. In developing-country populations, *G. lamblia* is ubiquitous and the initial infection is acquired in the first few weeks of life [27, 66, 118, 120, 123, 152, 153]. In developing-country settings, the initial or first few *G. lamblia* infections may result in diarrhea [74, 123] but immunity is rapidly acquired, thereupon conferring protection against symptomatic disease when subsequently exposed. *Giardia lamblia* gastroenteritis outbreaks in daycare centers in Canada provide indirect support for this explanation [18, 154]. Children of Canadian origin and those from other industrialized countries were more likely to be infected and to develop *Giardia* illness compared with children of immigrant families from developing countries [18, 154].

One well-established mechanism by which infants and young children in developing countries are protected against symptomatic disease upon exposure to *Giardia* is by suckling on mothers whose breast milk contains high titers of anti-*Giardia* secretory immunoglobulin A (SIgA). Breastfeeding is strongly associated with protection against clinical *Giardia* diarrhea, even though it does not generally prevent acquisition of *G. lamblia* infection or chronic carriage [152, 155]. Importantly, clinical protection is correlated with levels of specific anti-*G. lamblia* SIgA in milk [156]. Analogous evidence derives from experimental challenges of adult US volunteers [14]. Secretory IgA anti-*Giardia* antibodies were detected in duodenal fluids of subjects who experienced diarrhea following initial challenge with *Giardia* strain Gsm and these SIgA antibodies correlated with protection against clinical disease when the subjects were rechallenged but not with prevention of reinfection. There are also reports of anti-*Giardia* properties of breast milk due to moieties other than specific SIgA [157, 158]. Breast milk–derived passive protection may allow the child to acquire active immunity upon exposure to *G. lamblia* without paying the price of a clinically overt initial infection.

Another possible explanation for the apparent divergent clinical responses to *Giardia* in industrialized vs developing country pediatric populations may reside in differences in the small intestine. Young children in industrialized countries harbor low numbers of bacteria in their proximal small intestine and their mucosal architecture is characterized by elongated villi and modest numbers of intraepithelial and lamina propria lymphocytes. In contrast, the "normal" small intestine of young children living in impoverished, fecally contaminated conditions in developing countries is marked by blunted villi and hypercellularity of the lamina propria and by small bowel bacterial overgrowth [159–162]. While there is a spectrum of severity of such changes, they are collectively referred to as "environmental enteropathy" (or "tropical enteropathy") [159–162]. When the small intestine of the young child in the industrialized country setting is exposed to *G. lamblia,* acute diarrhea or other symptomatology not uncommonly results. In contrast, among young children in developing countries who often manifest environmental enteropathy, *G. lamblia* appears more often to result in asymptomatic colonization without acute diarrhea. In the environmental enteropathy gut, *G. lamblia* may modulate the innate immune system and mucosal environment such that a degree of protection is conferred against diarrhea caused by other enteropathogens. In vitro studies show that intestinal mucus may affect *Giardia* activity [163], and studies in mice suggest that the normal gut flora may play a role in susceptibility to *Giardia* infection [164]. If this phenomenon is also true in humans, it is possible that these factors might affect the clinical presentation of *Giardia* infection.

Whereas our systematic review did not find an association between *G. lamblia* infection and increased risk of acute diarrhea in children in developing-country settings, *Giardia* was significantly associated with persistent diarrhea in these pediatric populations [106, 107, 109]. The clinical illness of patients with giardiasis in industrialized settings who were infected during outbreaks or during travel to endemic areas also shows that symptoms may persist for several weeks [21, 154, 165, 166].

One must ponder why *G. lamblia* appears to be associated with a 3-fold increase in the risk of persistent diarrhea among children in developing countries but the pathogen is not associated with an increased risk of acute diarrhea. One hypothesis is that the infants and young children who develop persistent *G. lamblia* diarrhea constitute a subset of high-risk pediatric hosts because they have more severe chronic undernutrition than their peers of the same age (usually manifest as severe stunting), more severe environmental enteropathy or due to a genetic predisposition (such as combined IgA and immunoglobulin G2 deficiency).

Attributes of the parasite may also account for the propensity to cause persistent diarrhea in children in developing countries. Preliminary evidence supports an association between *G. lamblia* genotype A and B in the development of clinically overt diarrhea and other gastrointestinal symptoms

[80, 105, 142, 143, 148]. Antigenic variation manifested by *Giardia* may also play a role in the outcome or course of infection. *Giardia lamblia* trophozoites have variant specific proteins that coat the entire parasite including its flagella. Trophozoites can switch these proteins every 6.5–13.5 generations and this may allow evasion of the immune response, establishment of more persistent infection [36, 167–171], and a propensity to persistent diarrhea.

This systematic review supports the contention that asymptomatic *G. lamblia* infection somehow protects against diarrheal illness, although mechanistically it is not obvious how this occurs. *Giardia lamblia* infection triggers both host innate [36–38] and adaptive immune responses [36–38, 172]. Secretion of innate antimicrobial products having anti-*Giardia* activity (eg, defensin, lactoferrin) by the intestinal epithelium [36–38] and nitric oxide and reactive oxygen species has been described [37, 38]. Secretion of mucins [36, 37] and glycoproteins of the intestinal mucus layer can reduce attachment of a broad range of pathogens to the mucosal surface [36]. These responses elicited by *Giardia* may negatively affect other pathogens in the gut. Thus, repetitive or prolonged *Giardia* trophozoite attachment to the intestinal epithelium for extended periods may render the mucosa unfavorable for the attachment of other enteropathogens. *Giardia lamblia* has also been shown to bind cholera enterotoxin [173] and heavy *Giardia muris* infection significantly diminishes the intestinal secretion stimulated by cholera toxin compared to mouse intestine without *Giardia* [174]. Thus, *Giardia* may offer protection against otherwise severe diarrhea caused by enterotoxigenic bacterial pathogens like *Vibrio cholerae* and enterotoxigenic *E. coli*. Finally, one report suggests that the severity of rotavirus gastroenteritis in Bedouin infants may have been significantly reduced in the presence of *Giardia lamblia* coinfection [29].

Giardia lamblia infection induces serum immunoglobulin M and intestinal SIgA anti-*Giardia* antibodies [14, 36–38, 172], of which local SIgA is considered the most important for controlling and clearing the infection [37, 38]. Interleukin 6 and T-dependent responses have also been described [36–38]. These anti-*Giardia* responses may contribute to nonspecific or cross-protection against other enteropathogens [152].

In summary, evidence does not incriminate *G. lamblia* as a cause of acute diarrhea in young children in developing countries but does suggest an important role of *G. lamblia* infection in persistent diarrhea in such populations. Statistically well-powered, controlled studies such as the Global Enteric Multicenter Study (GEMS) are needed to clarify the circumstances under which *G. lamblia* infection may be involved in the development of diarrheal disease. In 7 developing-country sites, GEMS will help address whether *Giardia* infections in early infancy are positively linked to moderate-to-severe diarrhea, whether some pediatric hosts (eg, more stunted) are more prone to develop persistent diarrhea, whether *Giardia* decreases the risk of acute diarrhea from other specific enteropathogens, and whether specific *Giardia* genotypes exhibit enhanced pathogenicity over other genotypes.

Notes

Financial support. This study was funded by the Bill & Melinda Gates Foundation (grant number 38874).

Supplement sponsorship. This article was published as part of the supplement entitled "The Global Enteric Multicenter Study (GEMS)," sponsored by the Bill & Melinda Gates Foundation.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Marquardt WC, Demaree RS, Grieve RB. Giardia and giardiosis. Parasitology and vector biology. 2nd ed. San Diego, CA: Academic Press, **2000**:89–96.
2. Adam RD. Biology of *Giardia lamblia*. Clin Microbiol Rev **2001**; 14:447–75.
3. Ankarklev J, Jerlstrom-Hultqvist J, Ringqvist E, Troell K, Svard SG. Behind the smile: cell biology and disease mechanisms of *Giardia* species. Nat Rev Microbiol **2010**; 8:413–22.
4. Farthing MJ. Giardiasis. Gastroenterol Clin North Am **1996**; 25:493–515.
5. Lane S, Lloyd D. Current trends in research into the waterborne parasite *Giardia*. Crit Rev Microbiol **2002**; 28:123–47.
6. Nakano T, Binka FN, Afari EA, et al. Survey of enteropathogenic agents in children with and without diarrhoea in Ghana. J Trop Med Hyg **1990**; 93:408–12.
7. Nimri LF, Meqdam M. Enteropathogens associated with cases of gastroenteritis in a rural population in Jordan. Clin Microbiol Infect **2004**; 10:634–9.
8. Yoder JS, Harral C, Beach MJ. Giardiasis surveillance—United States, 2006–2008. MMWR Surveill Summ **2010**; 59:15–25.
9. European Centre for Disease Prevention and Control. Annual epidemiological report on communicable diseases in Europe 2010. European Center for Disease Prevention and Control: Stockholm, Sweden, **2010**.
10. Lebwohl B, Deckelbaum RJ, Green PH. Giardiasis. Gastrointest Endosc **2003**; 57:906–13.
11. Katz DE, Heisey-Grove D, Beach M, Dicker RC, Matyas BT. Prolonged outbreak of giardiasis with two modes of transmission. Epidemiol Infect **2006**; 134:935–41.
12. al-Bwardy MA, Ramia S, al-Frayh AR, et al. Bacterial, parasitic and viral enteropathogens associated with diarrhoea in Saudi children. Ann Trop Paediatr **1988**; 8:26–30.
13. Khan MM, Iqbal J, Ghafoor A, Burney MI. Aetiologic agents of diarrhoeal diseases in hospitalised children in Rawalpindi, Pakistan. J Diarrhoeal Dis Res **1988**; 6:228–31.
14. Nash TE, Herrington DA, Losonsky GA, Levine MM. Experimental human infections with *Giardia lamblia*. J Infect Dis **1987**; 156:974–84.
15. Rendtorff RC, Holt CJ. The experimental transmission of human intestinal protozoan parasites. IV. Attempts to transmit *Endamoeba coli* and *Giardia lamblia* cysts by water. Am J Hyg **1954**; 60:327–38.
16. White KE, Hedberg CW, Edmonson LM, Jones DB, Osterholm MT, MacDonald KL. An outbreak of giardiasis in a nursing home with evidence for multiple modes of transmission. J Infect Dis **1989**; 160:298–304.

17. Shaw PK, Brodsky RE, Lyman DO, et al. A communitywide outbreak of giardiasis with evidence of transmission by a municipal water supply. Ann Intern Med **1977**; 87:426–32.
18. Keystone JS, Krajden S, Warren MR. Person-to-person transmission of *Giardia lamblia* in day-care nurseries. Can Med Assoc J **1978**; 119:241–2, 247–8.
19. Addiss DG, Davis JP, Roberts JM, Mast EE. Epidemiology of giardiasis in Wisconsin: increasing incidence of reported cases and unexplained seasonal trends. Am J Trop Med Hyg **1992**; 47:13–9.
20. Lopez CE, Dykes AC, Juranek DD, et al. Waterborne giardiasis: a communitywide outbreak of disease and a high rate of asymptomatic infection. Am J Epidemiol **1980**; 112:495–507.
21. Birkhead G, Vogt RL. Epidemiologic surveillance for endemic *Giardia lamblia* infection in Vermont. The roles of waterborne and person-to-person transmission. Am J Epidemiol **1989**; 129:762–8.
22. Black RE, Dykes AC, Sinclair SP, Wells JG. Giardiasis in day-care centers: evidence of person-to-person transmission. Pediatrics **1977**; 60:486–91.
23. Nygard K, Schimmer B, Sobstad O, et al. A large community outbreak of waterborne giardiasis-delayed detection in a non-endemic urban area. BMC Public Health **2006**; 6:141.
24. Stanton B, Silimperi DR, Khatun K, et al. Parasitic, bacterial and viral pathogens isolated from diarrhoeal and routine stool specimens of urban Bangladeshi children. J Trop Med Hyg **1989**; 92:46–55.
25. Nimri LF, Elnasser Z, Batchoun R. Polymicrobial infections in children with diarrhoea in a rural area of Jordan. FEMS Immunol Med Microbiol **2004**; 42:255–9.
26. Shetty N, Narasimha M, Raghuveer TS, Elliott E, Farthing MJ, Macaden R. Intestinal amoebiasis and giardiasis in southern Indian infants and children. Trans R Soc Trop Med Hyg **1990**; 84:382–4.
27. Hollm-Delgado MG, Gilman RH, Bern C, et al. Lack of an adverse effect of *Giardia intestinalis* infection on the health of Peruvian children. Am J Epidemiol **2008**; 168:647–55.
28. Loening WE, Coovadia YM, van den Ende J. Aetiological factors of infantile diarrhoea: a community-based study. Ann Trop Paediatr **1989**; 9:248–55.
29. Bilenko N, Levy A, Dagan R, Deckelbaum RJ, El-On Y, Fraser D. Does co-infection with *Giardia lamblia* modulate the clinical characteristics of enteric infections in young children? Eur J Epidemiol **2004**; 19:877–83.
30. Veenemans J, Mank T, Ottenhof M, et al. Protection against diarrhea associated with *Giardia intestinalis* is lost with multi-nutrient supplementation: a study in Tanzanian children. PLoS Negl Trop Dis **2011**; 5:e1158.
31. Abramson JH. WINPEPI (PEPI-for-Windows): computer programs for epidemiologists. Epidemiol Perspect Innov **2004**; 1:6.
32. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med **2002**; 21:1539–58.
33. Sterne JA, Egger M. Funnel plots for detecting bias in meta-analysis: guidelines on choice of axis. J Clin Epidemiol **2001**; 54:1046–55.
34. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ **1997**; 315:629–34.
35. Borenstein M, Hedges L, Higgins J, Rothstein H. Comprehensive meta-analysis version 2. Englewood, NJ: Biostat, **2005**.
36. Muller N, von Allmen N. Recent insights into the mucosal reactions associated with *Giardia lamblia* infections. Int J Parasitol **2005**; 35:1339–47.
37. Roxstrom-Lindquist K, Palm D, Reiner D, Ringqvist E, Svard SG. Giardia immunity—an update. Trends Parasitol **2006**; 22:26–31.
38. Eckmann L. Mucosal defences against *Giardia*. Parasite Immunol **2003**; 25:259–70.
39. Buret AG. Pathophysiology of enteric infections with *Giardia duodenalis*. Parasite **2008**; 15:261–5.
40. Cotton JA, Beatty JK, Buret AG. Host parasite interactions and pathophysiology in *Giardia* infections. Int J Parasitol **2011**; 41:925–33.
41. Gillin FD, Reiner DS, Boucher SE. Small-intestinal factors promote encystation of *Giardia lamblia* in vitro. Infect Immun **1988**; 56:705–7.
42. Rendtorff RC. The experimental transmission of human intestinal protozoan parasites. II. *Giardia lamblia* cysts given in capsules. Am J Hyg **1954**; 59:209–20.
43. Weniger BG, Blaser MJ, Gedrose J, Lippy EC, Juranek DD. An outbreak of waterborne giardiasis associated with heavy water runoff due to warm weather and volcanic ashfall. Am J Public Health **1983**; 73:868–72.
44. Isaac-Renton JL, Philion JJ. Factors associated with acquiring giardiasis in British Columbia residents. Can J Public Health **1992**; 83:155–8.
45. Hoque ME, Hope VT, Kjellstrom T, Scragg R, Lay-Yee R. Risk of giardiasis in Aucklanders: a case-control study. Int J Infect Dis **2002**; 6:191–7.
46. Hoque ME, Hope VT, Scragg R, Kjellstrom T. Children at risk of giardiasis in Auckland: a case-control analysis. Epidemiol Infect **2003**; 131:655–62.
47. Gray SF, Gunnell DJ, Peters TJ. Risk factors for giardiasis: a case-control study in Avon and Somerset. Epidemiol Infect **1994**; 113:95–102.
48. Dennis DT, Smith RP, Welch JJ, et al. Endemic giardiasis in New Hampshire: a case-control study of environmental risks. J Infect Dis **1993**; 167:1391–5.
49. Balcioglu IC, Limoncu E, Ertan P, Yereli K, Ozbilgin A, Onag A. Incidence of giardiasis among siblings in Turkey. Pediatr Int **2003**; 45:311–3.
50. Chute CG, Smith RP, Baron JA. Risk factors for endemic giardiasis. Am J Public Health **1987**; 77:585–7.
51. Porter JD, Gaffney C, Heymann D, Parkin W. Food-borne outbreak of *Giardia lamblia*. Am J Public Health **1990**; 80:1259–60.
52. Mintz ED, Hudson-Wragg M, Mshar P, Cartter ML, Hadler JL. Foodborne giardiasis in a corporate office setting. J Infect Dis **1993**; 167:250–3.
53. Meyers JD, Kuharic HA, Holmes KK. *Giardia lamblia* infection in homosexual men. Br J Vener Dis **1977**; 53:54–5.
54. Keystone JS, Keystone DL, Proctor EM. Intestinal parasitic infections in homosexual men: prevalence, symptoms and factors in transmission. Can Med Assoc J **1980**; 123:512–4.
55. Phillips SC, Mildvan D, William DC, Gelb AM, White MC. Sexual transmission of enteric protozoa and helminths in a venereal-disease-clinic population. N Engl J Med **1981**; 305:603–6.
56. Pakianathan MR, McMillan A. Intestinal protozoa in homosexual men in Edinburgh. Int J STD AIDS **1999**; 10:780–4.
57. Pereira MG, Atwill ER, Barbosa AP. Prevalence and associated risk factors for *Giardia lamblia* infection among children hospitalized for diarrhea in Goiania, Goias State, Brazil. Rev Inst Med Trop Sao Paulo **2007**; 49:139–45.
58. Coles CL, Levy A, Dagan R, Deckelbaum RJ, Fraser D. Risk factors for the initial symptomatic *Giardia* infection in a cohort of young Arab-Bedouin children. Ann Trop Paediatr **2009**; 29:291–300.
59. Mahmud MA, Chappell C, Hossain MM, Habib M, Dupont HL. Risk-factors for development of first symptomatic *Giardia* infection among infants of a birth cohort in rural Egypt. Am J Trop Med Hyg **1995**; 53:84–8.
60. Boontanom P, Mungthin M, Tan-Ariya P, Naaglor T, Leelayoova S. Epidemiology of giardiasis and genotypic characterization of *Giardia duodenalis* in preschool children of a rural community, central Thailand. Trop Biomed **2011**; 28:32–9.
61. Ratanapo S, Mungthin M, Soontrapa S, et al. Multiple modes of transmission of giardiasis in primary schoolchildren of a rural community, Thailand. Am J Trop Med Hyg **2008**; 78:611–5.
62. Sprong H, Caccio SM, van der Giessen JW. Identification of zoonotic genotypes of *Giardia duodenalis*. PLoS Negl Trop Dis **2009**; 3:e558.

63. Eligio-Garcia L, Cortes-Campos A, Jimenez-Cardoso E. Genotype of *Giardia* intestinalis isolates from children and dogs and its relationship to host origin. Parasitol Res **2005**; 97:1–6.
64. Monis PT, Andrews RH, Mayrhofer G, Ey PL. Genetic diversity within the morphological species *Giardia intestinalis* and its relationship to host origin. Infect Genet Evol **2003**; 3:29–38.
65. Moore G, Cross W, McGuire D, et al. Epidemic giardiasis at a ski resort. N Engl J Med **1969**; 281:402–27.
66. Flanagan PA. Giardia—diagnosis, clinical course and epidemiology. A review. Epidemiol Infect **1992**; 109:1–22.
67. Addy PA, Antepim G, Frimpong EH. Prevalence of pathogenic *Escherichia coli* and parasites in infants with diarrhoea in Kumasi, Ghana. East Afr Med J **2004**; 81:353–7.
68. Albert MJ, Faruque AS, Faruque SM, Sack RB, Mahalanabis D. Case-control study of enteropathogens associated with childhood diarrhea in Dhaka, Bangladesh. J Clin Microbiol **1999**; 37:3458–64.
69. Al-Gallas N, Bahri O, Bouratbeen A, Ben Haasen A, Ben Aissa R. Etiology of acute diarrhea in children and adults in Tunis, Tunisia, with emphasis on diarrheagenic *Escherichia coli*: prevalence, phenotyping, and molecular epidemiology. Am J Trop Med Hyg **2007**; 77:571–82.
70. Bodhidatta L, McDaniel P, Sornsakrin S, Srijan A, Serichantalergs O, Mason CJ. Case-control study of diarrheal disease etiology in a remote rural area in Western Thailand. Am J Trop Med Hyg **2010**; 83:1106–9.
71. Casalino M, Yusuf MW, Nicoletti M, et al. A two-year study of enteric infections associated with diarrhoeal diseases in children in urban Somalia. Trans R Soc Trop Med Hyg **1988**; 82:637–41.
72. Chatterjee BD, Thawani G, Sanyal SN. Etiology of acute childhood diarrhoea in Calcutta. Trop Gastroenterol **1989**; 10:158–66.
73. Echeverria P, Taylor DN, Lexsomboon U, et al. Case-control study of endemic diarrheal disease in Thai children. J Infect Dis **1989**; 159:543–8.
74. El-Hakim MA, El-Sahn A. Association of parasites and diarrhoea among children less than five years of age in a rural area in Egypt. J Egypt Public Health Assoc **1996**; 71:439–63.
75. Gambhir IS, Jaiswal JP, Nath G. Significance of *Cryptosporidium* as an aetiology of acute infectious diarrhoea in elderly Indians. Trop Med Int Health **2003**; 8:415–9.
76. Gascon J, Vargas M, Schellenberg D, et al. Diarrhea in children under 5 years of age from Ifakara, Tanzania: a case-control study. J Clin Microbiol **2000**; 38:4459–62.
77. Gassama A, Sow PS, Fall F, et al. Ordinary and opportunistic enteropathogens associated with diarrhea in Senegalese adults in relation to human immunodeficiency virus serostatus. Int J Infect Dis **2001**; 5:192–8.
78. Georges MC, Roure C, Tauxe RV, et al. Diarrheal morbidity and mortality in children in the Central African Republic. Am J Trop Med Hyg **1987**; 36:598–602.
79. Gracey M, Burke V, Robinson J. Patterns of intestinal infection in Australian Aboriginal children. Ann Trop Paediatr **1983**; 3:35–9.
80. Haque R, Mondal D, Karim A, et al. Prospective case-control study of the association between common enteric protozoal parasites and diarrhea in Bangladesh. Clin Infect Dis **2009**; 48:1191–7.
81. Hassan EM, el-Meneza SA, el-Rashidy Z, Rashad R, Rabie S, Fahmy SA. Detection of enteropathogens in diarrhoeal diseases among malnourished Egyptian infant and children. J Egypt Public Health Assoc **1989**; 64:461–74.
82. Huilan S, Zhen LG, Mathan MM, et al. Etiology of acute diarrhoea among children in developing countries: a multicentre study in five countries. Bull World Health Organ **1991**; 69:549–55.
83. Korzeniowski OM, Dantas W, Trabulsi LR, Guerrant RL. A controlled study of endemic sporadic diarrhoea among adult residents of southern Brazil. Trans R Soc Trop Med Hyg **1984**; 78:363–9.
84. Menon BS, Abdullah S, Mahamud F, et al. Low prevalence of *Cryptosporidium* parvum in hospitalized children in Kota Bharu, Malaysia. Southeast Asian J Trop Med Public Health **2001**; 32:319–22.
85. Mertens TE, Wijenayake R, Pinto MR, et al. Microbiological agents associated with childhood diarrhoea in the dry zone of Sri Lanka. Trop Med Parasitol **1990**; 41:115–20.
86. Mubashir M, Khan A, Baqai R, et al. Causative agents of acute diarrhoea in the first 3 years of life: hospital-based study. J Gastroenterol Hepatol **1990**; 5:264–70.
87. Nahrevanian H, Assmar M, Samin MG. Cryptosporidiosis among immunocompetent patients with gastroenteritis in Iran: a comparison with other enteropathogenic parasites. J Microbiol Immunol Infect **2007**; 40:154–6.
88. Ogunsanya TI, Rotimi VO, Adenuga A. A study of the aetiological agents of childhood diarrhoea in Lagos, Nigeria. J Med Microbiol **1994**; 40:10–4.
89. Opintan JA, Newman MJ, Ayeh-Kumi PF, et al. Pediatric diarrhea in southern Ghana: etiology and association with intestinal inflammation and malnutrition. Am J Trop Med Hyg **2010**; 83:936–43.
90. Orlandi PP, Magalhaes GF, Matos NB, et al. Etiology of diarrheal infections in children of Porto Velho (Rondonia, Western Amazon region, Brazil). Braz J Med Biol Res **2006**; 39:507–17.
91. Orlandi PP, Silva T, Magalhaes GF, et al. Enteropathogens associated with diarrheal disease in infants of poor urban areas of Porto Velho, Rondonia: a preliminary study. Mem Inst Oswaldo Cruz **2001**; 96:621–5.
92. Oyofo BA, Subekti D, Tjaniadi P, et al. Enteropathogens associated with acute diarrhea in community and hospital patients in Jakarta, Indonesia. FEMS Immunol Med Microbiol **2002**; 34:139–46.
93. Paniagua GL, Monroy E, Garcia-Gonzalez O, Alonso J, Negrete E, Vaca S. Two or more enteropathogens are associated with diarrhoea in Mexican children. Ann Clin Microbiol Antimicrob **2007**; 6:17.
94. Sallon S, el-Shawwa R, Khalil M, et al. Diarrhoeal disease in children in Gaza. Ann Trop Med Parasitol **1994**; 88:175–82.
95. Sanchez-Vega JT, Tay-Zavala J, Aguilar-Chiu A, et al. Cryptosporidiosis and other intestinal protozoan infections in children less than one year of age in Mexico City. Am J Trop Med Hyg **2006**; 75:1095–8.
96. Simango C, Dindiwe J. The aetiology of diarrhoea in a farming community in Zimbabwe. Trans R Soc Trop Med Hyg **1987**; 81: 552–3.
97. Ming ZF, Xi ZD, Dong CS, et al. Diarrhoeal disease in children less than one year of age at a children's hospital in Guangzhou, People's Republic of China. Trans R Soc Trop Med Hyg **1991**; 85:667–9.
98. Meng CY, Smith BL, Bodhidatta L, et al. Etiology of diarrhea in young children and patterns of antibiotic resistance in Cambodia. Pediatr Infect Dis J **2011**; 30:331–5.
99. Househam KC, Dove MG, Smith MS. Enteropathogens associated with acute infantile diarrhoea in Bloemfontein, South Africa. J Trop Pediatr **1987**; 33:287–8.
100. Ajjampur SS, Rajendran P, Ramani S, et al. Closing the diarrhoea diagnostic gap in Indian children by the application of molecular techniques. J Med Microbiol **2008**; 57(Pt 11):1364–8.
101. Na'was TE, Abo-Shehada MN. A study of the bacterial and parasitic causes of acute diarrhoea in northern Jordan. J Diarrhoeal Dis Res **1991**; 9:305–9.
102. Stintzing G, Mollby R, Habte D. Enterotoxigenic *Escherichia coli* and other enteropathogens in paediatric diarrhoea in Addis Ababa. Acta Paediatr Scand **1982**; 71:279–86.
103. Hoge CW, Echeverria P, Rajah R, et al. Prevalence of *Cyclospora* species and other enteric pathogens among children less than 5 years of age in Nepal. J Clin Microbiol **1995**; 33:3058–60.
104. Rajeshwari K, Jaggi N, Aggarwal V, Kalra KK, Mittal SK, Baveja U. Determinants of symptomatic giardiasis in childhood. Trop Gastroenterol **1996**; 17:70–6.

105. Haque R, Roy S, Kabir M, Stroup SE, Mondal D, Houpt ER. Giardia assemblage A infection and diarrhea in Bangladesh. J Infect Dis **2005**; 192:2171–3.
106. Bhandari N, Bahl R, Dua T, Kumar R, Srivastava R. Role of protozoa as risk factors for persistent diarrhea. Indian J Pediatr **1999**; 66:21–6.
107. Sullivan PB, Marsh MN, Phillips MB, et al. Prevalence and treatment of giardiasis in chronic diarrhoea and malnutrition. Arch Dis Child **1991**; 66:304–6.
108. Yakoob J, Abbas Z, Beg MA, et al. Prevalences of *Giardia lamblia* and *Cryptosporidium parvum* infection in adults presenting with chronic diarrhoea. Ann Trop Med Parasitol **2010**; 104:505–10.
109. Mukhopadhyay C, Wilson G, Pradhan D, Shivananda PG. Intestinal protozoal infestation profile in persistent diarrhea in children below age 5 years in western Nepal. Southeast Asian J Trop Med Public Health **2007**; 38:13–9.
110. Guerrant RL, Kirchhoff LV, Shields DS, et al. Prospective study of diarrheal illnesses in northeastern Brazil: patterns of disease, nutritional impact, etiologies, and risk factors. J Infect Dis **1983**; 148:986–97.
111. Newman RD, Moore SR, Lima AA, Nataro JP, Guerrant RL, Sears CL. A longitudinal study of *Giardia lamblia* infection in north-east Brazilian children. Trop Med Int Health **2001**; 6:624–34.
112. Zaki AM, DuPont HL, el Alamy MA, et al. The detection of enteropathogens in acute diarrhea in a family cohort population in rural Egypt. Am J Trop Med Hyg **1986**; 35:1013–22.
113. Schorling JB, Wanke CA, Schorling SK, McAuliffe JF, de Souza MA, Guerrant RL. A prospective study of persistent diarrhea among children in an urban Brazilian slum. Patterns of occurrence and etiologic agents. Am J Epidemiol **1990**; 132:144–56.
114. Chunge RN, Nagelkerke N, Karumba PN, et al. Longitudinal study of young children in Kenya: intestinal parasitic infection with special reference to *Giardia lamblia*, its prevalence, incidence and duration, and its association with diarrhoea and with other parasites. Acta Trop **1991**; 50:39–49.
115. Molbak K, Wested N, Hojlyng N, et al. The etiology of early childhood diarrhea: a community study from Guinea-Bissau. J Infect Dis **1994**; 169:581–7.
116. Black RE, Lopez de Romana G, Brown KH, Bravo N, Bazalar OG, Kanashiro HC. Incidence and etiology of infantile diarrhea and major routes of transmission in Huascar, Peru. Am J Epidemiol **1989**; 129:785–99.
117. Baqui AH, Sack RB, Black RE, et al. Enteropathogens associated with acute and persistent diarrhea in Bangladeshi children less than 5 years of age. J Infect Dis **1992**; 166:792–6.
118. Hasan KZ, Pathela P, Alam K, et al. Aetiology of diarrhoea in a birth cohort of children aged 0–2 year(s) in rural Mirzapur, Bangladesh. J Health Popul Nutr **2006**; 24:25–35.
119. Kaminsky RG. Parasitism and diarrhoea in children from two rural communities and marginal barrio in Honduras. Trans R Soc Trop Med Hyg **1991**; 85:70–3.
120. Valentiner-Branth P, Steinsland H, Fischer TK, et al. Cohort study of Guinean children: incidence, pathogenicity, conferred protection, and attributable risk for enteropathogens during the first 2 years of life. J Clin Microbiol **2003**; 41:4238–45.
121. Boeke CE, Mora-Plazas M, Forero Y, Villamor E. Intestinal protozoan infections in relation to nutritional status and gastrointestinal morbidity in Colombian school children. J Trop Pediatr **2010**; 56:299–306.
122. Fraser D, Bilenko N, Deckelbaum RJ, Dagan R, El-On J, Naggan L. *Giardia lamblia* carriage in Israeli Bedouin infants: risk factors and consequences. Clin Infect Dis **2000**; 30:419–24.
123. Fraser D, Dagan R, Naggan L, et al. Natural history of *Giardia lamblia* and *Cryptosporidium* infections in a cohort of Israeli Bedouin infants: a study of a population in transition. Am J Trop Med Hyg **1997**; 57:544–9.
124. Shah N, DuPont HL, Ramsey DJ. Global etiology of travelers' diarrhea: systematic review from 1973 to the present. Am J Trop Med Hyg **2009**; 80:609–14.
125. Riddle MS, Sanders JW, Putnam SD, Tribble DR. Incidence, etiology, and impact of diarrhea among long-term travelers (US military and similar populations): a systematic review. Am J Trop Med Hyg **2006**; 74:891–900.
126. Black RE. Pathogens that cause travelers' diarrhea in Latin America and Africa. Rev Infect Dis **1986**; 8(suppl 2):S131–5.
127. Back E, Jonsson M, Wadstrom T. Enterotoxin-producing bacteria stools from Swedish United Nations soldiers in Cyprus. Infection **1978**; 6:116–20.
128. Shlim DR, Hoge CW, Rajah R, Rabold JG, Echeverria P. Is *Blastocystis hominis* a cause of diarrhea in travelers? A prospective controlled study in Nepal. Clin Infect Dis **1995**; 21:97–101.
129. Boggild AK, Yohanna S, Keystone JS, Kain KC. Prospective analysis of parasitic infections in Canadian travelers and immigrants. J Travel Med **2006**; 13:138–44.
130. Paschke C, Apelt N, Fleischmann E, et al. Controlled study on enteropathogens in travellers returning from the tropics with and without diarrhoea. Clin Microbiol Infect **2011**; 17:1194–200.
131. Pandey P, Bodhidatta L, Lewis M, et al. Travelers' diarrhea in Nepal: an update on the pathogens and antibiotic resistance. J Travel Med **2011**; 18:102–8.
132. DuPont HL, Olarte J, Evans DG, Pickering LK, Galindo E, Evans DJ. Comparative susceptibility of Latin American and United States students to enteric pathogens. N Engl J Med **1976**; 295:1520–1.
133. Brodsky RE, Spencer HC Jr, Schultz MG. Giardiasis in American travelers to the Soviet Union. J Infect Dis **1974**; 130:319–23.
134. Bolivar R, Conklin RH, Vollet JJ, et al. Rotavirus in travelers' diarrhea: study of an adult student population in Mexico. J Infect Dis **1978**; 137:324–7.
135. Echeverria P, Blacklow NR, Zipkin C, et al. Etiology of gastroenteritis among Americans living in the Philippines. Am J Epidemiol **1979**; 109:493–501.
136. Hoge CW, Shlim DR, Rajah R, et al. Epidemiology of diarrhoeal illness associated with coccidian-like organism among travellers and foreign residents in Nepal. Lancet **1993**; 341:1175–9.
137. Gascon J, Vargas M, Quinto L, Corachan M, Jimenez de Anta MT, Vila J. Enteroaggregative *Escherichia coli* strains as a cause of traveler's diarrhea: a case-control study. J Infect Dis **1998**; 177:1409–12.
138. Schultsz C, van den Ende J, Cobelens F, et al. Diarrheagenic *Escherichia coli* and acute and persistent diarrhea in returned travelers. J Clin Microbiol **2000**; 38:3550–4.
139. Andersson T, Forssell J, Sterner G. Outbreak of giardiasis: effect of a new antiflagellate drug, tinidazole. Br Med J **1972**; 2:449–51.
140. Merson MH, Morris GK, Sack DA, et al. Travelers' diarrhea in Mexico. A prospective study of physicians and family members attending a congress. N Engl J Med **1976**; 294:1299–305.
141. Cevallos A, Carnaby S, James M, Farthing JG. Small intestinal injury in a neonatal rat model of giardiasis is strain dependent. Gastroenterology **1995**; 109:766–73.
142. Read C, Walters J, Robertson ID, Thompson RC. Correlation between genotype of *Giardia duodenalis* and diarrhoea. Int J Parasitol **2002**; 32:229–31.
143. Aydin AF, Besirbellioglu BA, Avci IY, Tanyuksel M, Araz E, Pahsa A. Classification of *Giardia duodenalis* parasites in Turkey into groups A and B using restriction fragment length polymorphism. Diagn Microbiol Infect Dis **2004**; 50:147–51.
144. Al-Mohammed HI. Genotypes of *Giardia intestinalis* clinical isolates of gastrointestinal symptomatic and asymptomatic Saudi children. Parasitol Res **2011**; 108:1375–81.
145. Ajjampur SS, Sankaran P, Kannan A, et al. *Giardia duodenalis* assemblages associated with diarrhea in children in South India identified by PCR-RFLP. Am J Trop Med Hyg **2009**; 80:16–9.

146. Molina N, Minvielle M, Grenovero S, Salomon C, Basualdo J. High prevalences of infection with *Giardia intestinalis* genotype B among children in urban and rural areas of Argentina. Ann Trop Med Parasitol **2011**; 105:299–309.
147. Paintlia AS, Descoteaux S, Spencer B, et al. *Giardia lamblia* groups A and B among young adults in India. Clin Infect Dis **1998**; 26:190–1.
148. Sahagun J, Clavel A, Goni P, et al. Correlation between the presence of symptoms and the *Giardia duodenalis* genotype. Eur J Clin Microbiol Infect Dis **2008**; 27:81–3.
149. Homan WL, Mank TG. Human giardiasis: genotype linked differences in clinical symptomatology. Int J Parasitol **2001**; 31:822–6.
150. Kohli A, Bushen OY, Pinkerton RC, et al. *Giardia duodenalis* assemblage, clinical presentation and markers of intestinal inflammation in Brazilian children. Trans R Soc Trop Med Hyg **2008**; 102:718–25.
151. Lebbad M, Petersson I, Karlsson L, et al. Multilocus genotyping of human *Giardia* isolates suggests limited zoonotic transmission and association between assemblage B and flatulence in children. PLoS Negl Trop Dis **2011**; 5:e1262.
152. Mahmud MA, Chappell CL, Hossain MM, Huang DB, Habib M, DuPont HL. Impact of breast-feeding on *Giardia lamblia* infections in Bilbeis, Egypt. Am J Trop Med Hyg **2001**; 65:257–60.
153. Miotti PG, Gilman RH, Santosham M, Ryder RW, Yolken RH. Age-related rate of seropositivity of antibody to *Giardia lamblia* in four diverse populations. J Clin Microbiol **1986**; 24:972–5.
154. Wright RA, Spencer HC, Brodsky RE, Vernon TM. Giardiasis in Colorado: an epidemiologic study. Am J Epidemiol **1977**; 105:330–6.
155. Morrow AL, Reves RR, West MS, Guerrero ML, Ruiz-Palacios GM, Pickering LK. Protection against infection with *Giardia lamblia* by breast-feeding in a cohort of Mexican infants. J Pediatr **1992**; 121:363–70.
156. Walterspiel JN, Morrow AL, Guerrero ML, Ruiz-Palacios GM, Pickering LK. Secretory anti-*Giardia lamblia* antibodies in human milk: protective effect against diarrhea. Pediatrics **1994**; 93:28–31.
157. Gillin FD, Reiner DS, Wang CS. Human milk kills parasitic intestinal protozoa. Science **1983**; 221:1290–2.
158. Hernell O, Ward H, Blackberg L, Pereira ME. Killing of *Giardia lamblia* by human milk lipases: an effect mediated by lipolysis of milk lipids. J Infect Dis **1986**; 153:715–20.
159. Neto UF, Martins MCD, Lima FLD, Patricio FRS, Toledo MRF. Asymptomatic environmental enteropathy among slum-dwelling infants. J Am Coll Nutr **1994**; 13:51–6.
160. Anderson CM, Langford RF. Bacterial content of small intestine of children in health, in coeliac disease, and in fibrocystic disease of pancreas. Br Med J **1958**; 1:803–6.
161. Fagundes-Neto U, Viaro T, Wehba J, Patricio FR, Machado NL. Tropical enteropathy (environmental enteropathy) in early childhood: a syndrome caused by contaminated environment. J Trop Pediatr **1984**; 30:204–9.
162. dos Reis JC, de Morais MB, Oliva CA, Fagundes-Neto U. Breath hydrogen test in the diagnosis of environmental enteropathy in children living in an urban slum. Dig Dis Sci **2007**; 52:1253–8.
163. Zenian A, Gillin FD. Interactions of *Giardia lamblia* with human intestinal mucus: enhancement of trophozoite attachment to glass. J Protozool **1985**; 32:664–8.
164. Singer SM, Nash TE. The role of normal flora in *Giardia lamblia* infections in mice. J Infect Dis **2000**; 181:1510–2.
165. Hanevik K, Hausken T, Morken MH, et al. Persisting symptoms and duodenal inflammation related to *Giardia duodenalis* infection. J Infect **2007**; 55:524–30.
166. Kent GP, Greenspan JR, Herndon JL, et al. Epidemic giardiasis caused by a contaminated public water supply. Am J Public Health **1988**; 78:139–43.
167. Prucca CG, Rivero FD, Lujan HD. Regulation of antigenic variation in *Giardia lamblia*. Annu Rev Microbiol **2011**; 65:611–30.
168. Nash TE, Herrington DA, Levine MM, Conrad JT, Merritt JW Jr. Antigenic variation of *Giardia lamblia* in experimental human infections. J Immunol **1990**; 144:4362–9.
169. Nash TE. Surface antigenic variation in *Giardia lamblia*. Mol Microbiol **2002**; 45:585–90.
170. Nash TE, Lujan HT, Mowatt MR, Conrad JT. Variant-specific surface protein switching in *Giardia lamblia*. Infect Immun **2001**; 69:1922–3.
171. Singer SM, Elmendorf HG, Conrad JT, Nash TE. Biological selection of variant-specific surface proteins in *Giardia lamblia*. J Infect Dis **2001**; 183:119–24.
172. Ali SA, Hill DR. *Giardia intestinalis*. Curr Opin Infect Dis **2003**; 16:453–60.
173. McCardell BA, Madden JM, Stanfield JT, Tall BD, Stephens MJ. Binding of cholera toxin to *Giardia lamblia*. J Clin Microbiol **1987**; 25:1786–8.
174. Ljungstrom I, Holmgren J, Svennerholm AM, Ferrante A. Changes in intestinal fluid and mucosal immune responses to cholera toxin in *Giardia muris* infection and binding of cholera toxin to *Giardia muris* trophozoites. Infect Immun **1985**; 50:243–9.