

## Risk factors for the initial symptomatic giardia infection in a cohort of young Arab-Bedouin children

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### Abstract

**Background:** Giardiasis is a common protozoan infection with clinical manifestations in children ranging from asymptomatic carriage to persistent diarrhoea with malabsorption. It can lead to growth and developmental retardation.

**Aim:** The study evaluated risk factors for the initial symptomatic giardiasis (SG) episode among Arab-Bedouin children in Israel.

**Methods:** A community-based, prospective cohort study was conducted in Rahat, a Bedouin township in southern Israel. Infants ( $n=238$ ) were followed by weekly visits from birth to age 18 months. Giardia infection was identified by antigen detection in faecal specimens.

**Results:** Approximately 26% of children experienced one or more SG episode. Mean (SD) age for first SG episode was 12.3 (3.3) months, with 95% of episodes occurring in children >6 months of age. Risk for the first SG in children >6 months of age was associated with it being spring or summer [odds ratio (OR) 6.16,  $p<0.001$ ], exposure to livestock (OR 4.89,  $p=0.002$ ) and prior infection with entero-aggregative *Escherichia coli* (EAEC) (OR 1.12 for each additional percentage in stool prevalence,  $p=0.02$ ). Weight-for-age Z-scores at age 6 months were inversely related to SG risk (OR 0.62 for each unit increase in Z-score,  $p=0.029$ ).

**Conclusions:** Giardiasis is an important cause of diarrhoea in Bedouin children. Increased risk of SG in spring/summer might be linked to environmental conditions or seasonal dietary practices which increase virulence or transmission. SG in those exposed to livestock suggests that there are zoonotic risk factors or that hygiene is a causal factor. The association between EAEC infection and SG warrants further investigation.

### Introduction

Giardiasis is the most common protozoan infection in children worldwide.<sup>1,2</sup> In developing countries, the parasite is prevalent among young children and repeat infections are common in the 1st 2 years of life.<sup>3–5</sup> The presentation of giardiasis is highly variable,

ranging from asymptomatic carriage to chronic diarrhoea with severe malabsorption and malnutrition.<sup>6</sup> The majority of infections are asymptomatic, yet carriers can continue to excrete cysts and transmit infections for more than a year.<sup>7–9</sup> Symptomatic giardiasis (SG) is more common in infants and children than in adults. Between 25 and 50% of all *Giardia lamblia* (GL) infections are symptomatic and are characterised in children by a range of symptoms which include watery diarrhoea, vomiting, belching, lack of weight gain and anaemia.<sup>10,11</sup> These infections can progress

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to chronic or persistent diarrhoea with protein and fat malabsorption, leading to vitamin A and B<sub>12</sub> deficiency and weight loss of up to 20% of ideal body weight<sup>12-14</sup> along with growth and developmental retardation.<sup>15,16</sup> In addition, SG is associated with decreased intestinal absorption of oral antibiotics which can lead to failure of treatment for concurrent infections.<sup>17</sup>

GL is endemic in the Negev region of southern Israel and although its population is primarily Jews from Eastern Europe and North Africa, it is the Arab-Bedouin population there who are at greater risk of GL infection. Results from an earlier longitudinal study showed that 88% of Bedouin children became GL carriers by the age of 2 years.<sup>3</sup> Compared with the generally westernised Jewish population of the Negev region, the Bedouin are often of low socio-economic status and are in transition from a semi-nomadic to a more settled lifestyle. Bedouin children share many characteristics with children from other non-industrialised populations around the world, including poor nutritional status and a high incidence of infectious diseases.<sup>18,19</sup>

It is unclear why some children are prone to SG. Data from several studies suggest that size of infectious dose, strain variation, undernutrition, lack of breast-feeding, secretory IgA deficiency and contact with domestic animals might play roles in the establishment of SG in children.<sup>20-23</sup> Yet, few data have been published on the factors which predispose children to the initial SG episode. Identification of modifiable factors for the first episode is needed in order to develop effective strategies to prevent severe SG in infants and, in turn, avert the potential malabsorption-malnutrition cycle that can lead to growth and developmental delays in children in vulnerable populations. We evaluated risk factors for the initial symptomatic GL infection in a cohort of Bedouin children recruited at birth and followed until 18 months of age.

## Subjects and Methods

### *Subjects and setting*

The study methods have been described previously.<sup>3</sup> Briefly, a prospective cohort study was conducted in the Bedouin township of Rahat, the largest Bedouin settlement in the Negev region of southern Israel, to evaluate risk factors for diarrhoeal illness in Arab-Bedouin children. From December 1994 to March 1997, we recruited families of Bedouin newborns from Rahat at Soroka University Medical Center (SUMC) in the nearby city of Beer Sheva. More than 99% of Rahat women deliver at Soroka hospital, which is the only in-patient tertiary care facility in the Negev. Enrolment was restricted to infants weighing >2500 g at birth and who had no conditions which would affect normal feeding and growth. We randomised the day of the week on which recruitment began and newborns were recruited from the 125-150 infants born to Rahat women each week to ensure that enrolment reflected the temporal distribution of births to Rahat women throughout the year. During this period, 247 healthy newborns were enrolled following receipt of informed consent. Ethical approval for the study was granted by the Institutional Review Boards at Soroka University Medical Center/Ben-Gurion University of the Negev, Beer Sheva, Israel and Columbia Presbyterian Medical Center, New York.

### *Data collection*

Upon enrolment, a trained interviewer ascertained data on family socio-demographic and household characteristics. Birthweight was obtained from hospital records. At weekly home visits, information on the child's history of illness, breast-feeding status, weaning foods and the health of family members during the previous 7 days was collected by interviewers using standardised questionnaires and probes. These were conducted until the child reached 18 months of age. Weight and

length were measured by trained public health nurses during a visit to Well Child Clinics (Israeli Ministry of Health) for immunisation and which was not related to illness. Height was measured to the nearest 0.1 cm and weight to the nearest 10 g, according to recommendations by the World Health Organization. Scales and measuring boards were validated regularly. The environmental and physical condition of the home and property, water availability, the presence of indoor toilet facilities and the presence of livestock were assessed when the child was aged 3 months.

The children's stools were collected for viral and parasitological studies by mothers at 14-day intervals into tubes containing phenol-alcohol-formaldehyde and refrigerated. Mothers also obtained faecal swabs for bacterial studies, the swabs were stored in transport media at room temperature. Within 48 hours of collection, stool samples and faecal swabs were transported to the laboratories at Soroka University Medical Center.

#### *Definition and ascertainment of symptomatic giardiasis*

Diarrhoeal episodes were recorded by study interviewers during weekly home visits. Children with diarrhoea were referred to their local health clinics for treatment. An episode of diarrhoea was defined as when a mother reported her child passing three or more liquid or semi-liquid stools in a 24-hour period, except for infants <1 month of age, for whom the definition was four or more liquid or semi-liquid stools in a 24-hour period. An episode ended when the child experienced 3 consecutive diarrhoea-free days. Given that the median incubation period for SG is 7–10 days,<sup>24,25</sup> a case was defined as being a child with diarrhoea whose faecal specimen tested positive for GL by antigen detection 3–14 days prior to onset of symptoms with no evidence of infection by any other enteric pathogen.

#### *Laboratory procedures*

*Stool analysis.* When a diarrhoeal episode occurred, stool samples were collected immediately and at weekly intervals until 1 week after the episode had ended. All faecal samples underwent parasitological, viral and bacterial testing to detect the presence of enteric pathogens. The parasites *Cryptosporidium* and *Giardia lamblia* were identified using ELISA (ProSpecT *Cryptosporidium* Microplate Assay, ProSpecT *Giardia* Microplate Assay, Alexon-Trend, Ramsey, MI, USA). Bacteriological testing was performed on all faecal swabs to identify *Campylobacter* spp, *Shigella* spp and *Salmonella* by routine laboratory methods. All diarrhoea samples and a 30% sample of non-diarrhoeal stools were tested by DNA hybridisation with radio-active probes to identify the following *Escherichia coli* strains: diffuse adherent *E. coli* (DAEC), entero-aggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC) and enteroinvasive *E. coli* (EIEC). The presence of rotavirus strains in these stools was detected by ELISA (reagents by Dako, Copenhagen, Denmark). For each pathogen, carriage prevalence was calculated as the number of faecal specimens which tested positive for each pathogen divided by the number of times faecal specimens were collected from each child from birth until 15 days before the first episode of SG. Proportions were used because the number of faecal specimens collected depended on the frequency of diarrhoea experienced by each child during follow-up.

#### *Statistical analysis*

We evaluated the association between the first SG episode and a range of variables whose occurrence preceded the onset of the initial symptomatic GL infection by 15 days or more: (i) host characteristics (gender, birthweight), (ii)

demographic/socio-economic indicators (maternal age and years of formal education, dwelling type, presence of a refrigerator, number of children <5 years in the household), (iii) nutrition-related factors (anthropometric status at age 6 months, breastfeeding status, duration of exclusive breastfeeding), (iv) environmental details (location of water source and toilet, presence of domestic animals, cleanliness of infant, season of infection), and (v) prior enteric infections (cumulative proportion of test-positive faecal samples). Z-scores were calculated for anthropometric measures at 6 and 12 months of age (EpiInfo 6, Centers for Disease Control and Prevention, Atlanta, GA, USA) using the WHO/National Center for Health Statistics (NCHS) reference population data.<sup>26</sup> Univariate associations between each potential risk factor and case status were determined by two-tailed  $\chi^2$  or Fisher's exact tests, where appropriate (Stata 9.0, Stata Corporation, College Station, TX, USA). Logistic regression models were constructed to identify factors associated with the first symptomatic GL infection. Crude odds ratios and 95% confidence intervals were calculated to measure the association between individual exposures and risk for the first symptomatic episode of GL. Variables implicated in the literature, such as nutritional status, or statistically significant at the level of  $p < 0.1$  by univariate

analyses were included in the multiple logistic regression models. Odds ratios were used to estimate the independent effects of potential risk factors from these models. Associations between potential risk factors and SG were considered to be statistically significant at  $p < 0.05$  in the multiple logistic regression models.

## Results

### *Characteristics of the study population*

Nine of the 247 healthy newborns enrolled were withdrawn from the study because their families lived in remote, desert areas, or were absent from their household for extended periods and could not be contacted. Of the remaining 238 infants included, more than 95% (227/238) completed at least 70 of a total of 78 weeks of follow-up. Half of the children in the cohort were male (119/238). The majority of children (68%) were born in the autumn or winter months. Approximately 83% (198/238) of subjects had at least one sibling (Table 1). More than 81% (166/203) of the children's mothers had had some formal education and 61.6% (125/203) had completed primary school.

All children in the cohort were breastfed for some period. The mean (SD) duration of breastfeeding reported by mothers was 39.7 (22) weeks and there was considerable

TABLE 1. *Baseline characteristics of study infants (n=238).*

Characteristics	No. (%) Total=238
Male	119/238 (50.0)
Three or more siblings	198/238 (83.2)
Mothers with <1 yr education	78/203 (38.4)
Proportion of children breastfed	238/238 (100.0)
Underweight (weight-for-age Z-score <-2) at age 6 m	3/238 (1.3)
Dwelling made of concrete	202/238 (84.9)
Household connected to municipal water mains	222/238 (93.3)
Household exposure to livestock	22/238 (9.2)
Toilet in household	204/238 (85.7)
Family-owned refrigerator in household	209/238 (88.8)

variation in the length of breastfeeding, ranging from 2 to 75 weeks. However, no child was exclusively breastfed for more than 4 weeks. The mean (SD) weight-for-age Z-score was -0.53 (0.82) at age 6 months. The proportion of children who were underweight (weight-for-age Z-score  $\leq -2$ ) was low, 1.3% at age 6 months.

Approximately 96% (224/238) of the children lived in concrete houses and 93% (222/238) of the homes were connected to the municipal water supply. The proportion of households with indoor toilets was 85% (204/238). Less than 10% (22/238) of children lived in households with cattle, sheep, chickens or goats on the premises.

The mean (SD) number of diarrhoeal episodes experienced by participants up to 18 months of age was 2.6 (2.9). GL was detected in 25.8% (2437/9453) of all stools collected, and was the most frequently detected enteric pathogen. *Campylobacter* spp were isolated in 7.2% of all faecal swabs (680/9396). Mean (SD) rates of isolation of *E. coli* strains, DAEC 2.5% (2.9) and EAEC 2.4% (3.1), and rates of EPEC 0.6% (1.2), EHEC 0.1% (0.4) and ETEC 0.4% (1.0) were lower. Rates of isolation of *Cryptosporidium* 2.5% (2.8), rotavirus 0.7% (1.3), *Shigella* spp 0.5% (1.3) and *Salmonella* 0.3% (0.9) were also low. EIEC was not detected in any of the faecal specimens.

#### *Prevalence of symptomatic giardiasis*

Of the 238 children in the study, 89.4% (213) experienced at least one asymptomatic GL infection and 26% (62) experienced at least one episode of SG. Mean (SD) age at the first SG episode was 12.3 (3.3) months, with an age range of 3.5–17.3 months. Three cases occurred before the age of 6 months. In contrast, the mean (SD) age at the first asymptomatic infection was 9.5 (3.9) months, with 21.1% (50/238) of these occurring before the age of 6 months.

#### *Risk factors for symptomatic giardiasis in children aged 6–18 months*

The evaluation of risk was restricted to the 235 children over the age of 6 months because of the low incidence of SG in children younger than 6 months. We evaluated the association between first SG episode and season, demographic characteristics, nutritional status indicators (weight-for-age Z-score and underweight status at age 6 months, proportion of weeks the child was breastfed at least once, weeks of exclusive breastfeeding), environmental/household factors, and enteric infection at least 15 days before the onset of the first episode of SG.

In the univariate analysis, symptomatic infections were four times more common in the spring/summer months than in the autumn/winter months (Table 2). Lack of maternal education was associated with increased risk of symptomatic GL infection. The risk of symptomatic infection was doubled in participants with mothers who had received no school education compared with those with mothers with one or more years of education. In addition, children with three or more siblings had three times the risk of symptomatic infection than those without siblings. Neither the child's gender nor the mother's age was associated with the risk of SG infection. There were no statistically significant associations between proportion of weeks the child was breastfed at least once or weeks of exclusive breastfeeding and first symptomatic GL infection. Similarly, there was no apparent association between the risk of first symptomatic infection and nutritional status at 6 months of age as measured by weight-for-age Z-score or underweight status.

Among environmental factors, children whose families raised livestock were at significantly greater risk of SG than children whose families did not. The odds of SG were approximately three times greater in children exposed to chicken or cattle at household level than in children whose

TABLE 2. Crude odds ratios for selected risk factors for first symptomatic giardiasis episode in Bedouin children aged 6–18 months (n=235).

Risk factor	Univariate analysis		
	Odds ratio	95% confidence interval	p-value
Spring/summer season	4.33	2.32–78.08	<0.001
Mothers with <1 yr education	1.94	0.89–4.20	0.93
Three or more siblings	2.68	1.00–7.20	0.050
Household exposure to livestock	2.79	1.14–6.85	0.025
Weight-for-age Z score (age 6 m)*	0.83	0.58–1.20	0.317
Prior EAEC infection <sup>†‡</sup>	1.09	1.0–1.18	0.040
Prior asymptomatic <i>G. lamblia</i> infection <sup>§‡</sup>	0.98	0.96–1.00	0.062

\* For each additional unit increase in weight-for age Z-score; <sup>†</sup> for each additional percentage increase in proportion of all stools which tested positive for EAEC; <sup>‡</sup> infections occurring >15 days before initial symptomatic *G. lamblia* infection; <sup>§</sup> for each additional percentage increase in proportion of all stools which tested positive for *G. lamblia*.

families had no livestock on the premises. No other environmental factors were associated with SG infection.

Of the enteric pathogens assessed, two were found to be associated with SG. The odds of symptomatic infection were significantly greater in children with a history of EAEC infection. Prevalence rates for EAEC in stool ranged from 0 to 26.1% of the total stools collected. The risk of SG was correlated with EAEC stool prevalence. Conversely, previous asymptomatic infection with GL was found to reduce the risk of symptomatic infection. The isolation rates of asymptomatic GL were between 0 and 82.1%.

When the associations were evaluated using multiple logistic regression models, four were statistically significant. Children

were five times more likely to experience a clinical infection during the spring and summer months than in the autumn and winter months (Table 3). With regard to nutritional status at age 6 months, a unit increase in weight-for-age Z-score was associated with a 36% decrease in the odds of contracting SG. Household exposure to livestock was associated with a risk of infection four times greater than in children who were not exposed at home. A past history of isolation of faecal EAEC was also associated with an increased risk of symptomatic infection. Prior asymptomatic GL infection and a lack of maternal education were not statistically significant in the multivariate analysis. The Hosmer–Lemeshow goodness-of-fit test provided no evidence to reject the final multivariate model

TABLE 3. Risk factors for first symptomatic giardiasis episode in Bedouin children aged 6–18 months (n=235).

Risk factor	Multiple logistic regression analysis*		
	Odds ratio	95% confidence interval	p-value
Spring/summer season	5.43	2.77–10.70	<0.001
Household exposure to cattle/chickens	4.36	1.62–11.70	0.004
Weight-for-age Z-score (age 6 m) <sup>†</sup>	0.64	0.43–0.98	0.038
Prior EAEC infection <sup>‡¶</sup>	1.11	1.00–1.21	0.036

\* Hosmer–Lemeshow  $\chi^2(8)=10.08$ ,  $p=0.260$ ; <sup>†</sup> for each additional unit increase in weight-for-age Z-score; <sup>‡</sup> for each additional percentage increase in proportion of all stools which tested positive for EAEC; <sup>¶</sup> infections occurring  $\geq 15$  days before initial symptomatic *G. lamblia* infection

(Hosmer–Lemeshow  $\chi^2$  (8)=7.70,  $p=0.260$ ).

## Discussion

Symptomatic infection with GL is common among young Israeli Bedouin children. More than a quarter of the children in our cohort experienced one or more episodes of GL-associated diarrhoea before 18 months of age. Given that the incubation period for *G. lamblia* ranges between 3 and 25 days, our results are likely to underestimate the true incidence of symptomatic GL infection in this population.<sup>24,25</sup> On average, the first symptomatic episode occurred just after a child's 1st birthday, 3 months after his/her first asymptomatic infection. The age pattern is similar to that observed in a study conducted in a Brazilian urban slum.<sup>5</sup> In contrast, the greatest risk of symptomatic infection in Egypt was greatest in children under the age of 6 months.<sup>27</sup> The differences in age of first onset might be explained by the water storage practices in rural Egypt. To keep it cool, water from communal wells and taps is often stored in clay pots. The lower temperatures facilitate the survival of GL cysts, increasing the risk of GL infection. Unlike in rural Egypt, water in Brazil and Israel is piped directly into homes from municipal water mains and is therefore less likely to be a source of infection of young children. Alternatively, the differences in the age pattern of infection between Egyptian and Bedouin children might result from differences in socio-economic conditions. Improvements in socio-economic conditions at the population level have been shown to result in the risk of a number of infections, such as hepatitis A, moving to older age groups.<sup>28,29</sup>

The risk of symptomatic infection was greatest in the spring and summer months. This is consistent with evidence from several studies conducted among Bedouin children in southern Israel which show that diarrhoea and hospitalisations peak during the late

spring and summer months.<sup>19</sup> Previous studies in the Negev have shown no seasonality of asymptomatic infection among the Bedouins.<sup>8,30</sup> There is some evidence that GL of genotype assemblage A is more frequently associated with diarrhoea than GL of genotype assemblage B.<sup>31</sup> The incidence of parasitic infections has been linked to warmer, wetter weather.<sup>32</sup> One might speculate that factors confer a growth advantage to or result in higher transmission of the diarrhoeagenic genotype. Another possibility is that the increased risk of symptomatic infection is linked to seasonal variations in diet (e.g. preparation and consumption of cold foods which are more likely to become contaminated) or changes in sources of drinking water (e.g. greater reliance on drinking water from untreated surface water or wells versus municipal water).

We have identified an association between previous infection with EAEC, a common cause of persistent diarrhoea in infants,<sup>33</sup> and SG. EAEC was isolated from stool more frequently during spring and summer months. We did not find an association with other species of *E. coli* or with any other pathogen. Two previous studies found that co-infection with any one of several enteric pathogens, including EAEC, did not change symptoms or alter the course of GL infection.<sup>21,27</sup> However, this observation does not rule out the possibility that previous infection with EAEC could predispose to SG. The incubation period for EAEC is 20–48 hours. The short incubation of EAEC and the seasonal correlation between the two pathogens suggest that the risk of the first symptomatic GL infection rises sharply soon after EAEC infection. EAEC infection is characterised by triggering of inflammatory responses in the gastro-intestinal tract, including elevated levels of IL-8, IL1 $\beta$  and faecal lactoferrin, and production of toxins that disrupt intestinal absorptive function.<sup>33</sup> These alterations might contribute to undernutrition and lead to prolonged malabsorption, leaving children more

susceptible to other infections.<sup>34</sup> It is therefore possible that EAEC infection may demonstrate facilitative invasion of the intestine by a second pathogen. There is no documented evidence of any bacterial–protozoan relationship in the published literature. More studies are needed to determine if there is a causal association between the two enteropathogens.

Household exposure to chicken or cattle was also observed to increase the risk of symptomatic infection. GL has been detected in the stool of livestock.<sup>1</sup> While there has been no direct evidence of transmission of GL between animals and humans, several epidemiological studies have observed an association between animal exposure and SG.<sup>1,27,35</sup>

We found a strong negative association between weight-for-age *Z*-score at age 6 months and risk of SG infection. There is some evidence of an association between mild-to-moderate malnutrition and SG.<sup>5,27</sup> Future studies need to help define the role of nutritional factors, including micronutrients, on the clinical presentation of giardia infection.

A potential limitation of this study relates to environmental assessment of the household. Although the majority of houses were connected to the municipal water supply and had indoor toilets, we were unable to assess the extent to which these facilities were used. Anecdotal evidence suggests that access to tap water and the presence of indoor toilets are not correlated with use. Therefore, it is possible that household environmental factors might play an important role in the risk of GL infection among Bedouin children.

In summary, we found that *G. lamblia* is a common cause of diarrhoea in young children in a Bedouin community in southern Israel. Spring/summer season, household exposure to chickens and cattle, being underweight at the age of 6 months and prior infection with EAEC were identified as risk factors for the initial symptomatic infection. Given the endemicity of *G.*

*lamblia* in this area, mass treatment of giardiasis with anti-giardial chemotherapy is unlikely to lower the risk of symptomatic giardiasis.<sup>8,36</sup> It is possible that environmental conditions and seasonal dietary practices enhance virulence or transmission of infection during the spring/summer months. The elevated risk of SG among children exposed to domestic animals in the home suggests that either there are zoonotic risk factors for symptomatic infection or that hygiene is the casual factor. Efforts to educate in the practice of personal hygiene, especially related to safe drinking water, food preparation and animal contact, might help to reduce disease transmission and symptomatic infection. Future studies should investigate the potential zoonotic transmission of giardia and evaluate the relationship between this parasite and prior EAEC infection.

### Contributors

C. L. Coles analysed the data and wrote the initial and final manuscripts. A. Levy was the study co-ordinator and contributed to the design, collection of data and editing of manuscripts. R. Dagan supervised the microbiological aspects of the study and contributed to the microbiological analysis of the stool specimens, and editing the manuscripts. R. J. Deckelbaum and D. Fraser wrote the grant proposal and edited the manuscripts. D. Fraser contributed to the design, analysis and editing of the final manuscripts.

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## References

- 1 Thompson RC. Giardiasis as a re-emerging infectious disease and its zoonotic potential. *Int J Parasitol* 2000; **30**:1259–67.
- 2 Gardner TB, Hill DR. Treatment of giardiasis. *Clin Microbiol Rev* 2001; **14**:114–28.
- 3 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.
- 4 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.
- 5 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.
- 6 Flanagan PA. *Giardia*—diagnosis, clinical course and epidemiology. A review. *Epidemiol Infect* 1992; **109**:1–22.
- 7 Farthing MJ. Giardiasis. *Gastroenterol Clin North Am* 1996; **25**:493–515.
- 8 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.
- 9 Ish-Horowitz M, Korman SH, Shapiro M, *et al.* Asymptomatic giardiasis in children. *Pediatr Infect Dis J* 1989; **8**:773–9.
- 10 Hill DR. Giardiasis. Issues in diagnosis and management. *Infect Dis Clin North Am* 1993; **7**:503–25.
- 11 Wolfe MS. Giardiasis. *Clin Microbiol Rev* 1992; **5**:93–100.
- 12 Farthing MJ, Mata L, Urrutia JJ, Kronmal RA. Natural history of *Giardia* infection of infants and children in rural Guatemala and its impact on physical growth. *Am J Clin Nutr* 1986; **43**:395–405.
- 13 Lengerich EJ, Addiss DG, Juranek DD. Severe giardiasis in the United States. *Clin Infect Dis* 1994; **18**:760–3.
- 14 Thompson RC, Reynoldson JA, Mendis AH. *Giardia* and giardiasis. *Adv Parasitol* 1993; **32**:71–160.
- 15 Petri WA Jr, Miller M, Binder HJ, Levine MM, Dillingham R, Guerrant RL. Enteric infections, diarrhea, and their impact on function and development. *J Clin Invest* 2008; **118**:1277–90.
- 16 Berkman DS, Lescano AG, Gilman RH, Lopez SL, Black MM. Effects of stunting, diarrhoeal disease, and parasitic infection during infancy on cognition in late childhood: a follow-up study. *Lancet* 2002; **359**:564–71.
- 17 Craft JC, Holt EA, Tan SH. Malabsorption of oral antibiotics in humans and rats with giardiasis. *Pediatr Infect Dis J* 1987; **6**:832–6.
- 18 Forman MR, Guptill KS, Chang DN, *et al.* Undernutrition among Bedouin Arab infants: the Bedouin Infant Feeding Study. *Am J Clin Nutr* 1990; **51**:343–9.
- 19 Levy A, Fraser D, Vardi H, Dagan R. Hospitalizations for infectious diseases in Jewish and Bedouin children in southern Israel. *Eur J Epidemiol* 1998; **14**:179–86.
- 20 Mahmud MA, Chappell CL, Hossain MM, Huang DB, Habib M, DuPont HL. Impact of breastfeeding on *Giardia lamblia* infections in Bilbeis, Egypt. *Am J Trop Med Hyg* 2001; **65**:257–60.
- 21 Rajeshwari K, Jaggi N, Aggarwal V, Kalra KK, Mittal SK, Baveja U. Determinants of symptomatic giardiasis in childhood. *Trop Gastroenterol* 1996; **17**:70–6.
- 22 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.
- 23 Perez O, Lastre M, Bandera F, *et al.* Evaluation of the immune response in symptomatic and asymptomatic human giardiasis. *Arch Med Res* 1994; **25**:171–7.
- 24 Pickering LK, Engelkirk PG. *Giardia lamblia*. *Pediatr Clin North Am* 1988; **35**:565–77.
- 25 Walterspiel JN, Pickering LK. *Giardia* and giardiasis. *Prog Clin Parasitol* 1994; **4**:1–26.
- 26 Dibley MJ, Staehling N, Nieburg P, Trowbridge FL. Interpretation of Z-score anthropometric indicators derived from the international growth reference. *Am J Clin Nutr* 1987; **46**:749–62.
- 27 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.
- 28 Green MS, Aharonowitz G, Shohat T, Levine R, Anis E, Slater PE. The changing epidemiology of viral hepatitis A in Israel. *Isr Med Assoc J* 2001; **3**:347–51.
- 29 Tanaka J. Hepatitis A shifting epidemiology in Latin America. *Vaccine* 2000; **18** (suppl 1):S57–60.
- 30 Farthing MJ. *Giardia* comes of age: progress in epidemiology, immunology and chemotherapy. *J Antimicrob Chemother* 1992; **30**:563–6.
- 31 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.
- 32 Pawlowski SW, Warren CA, Guerrant R. Diagnosis and treatment of acute or persistent diarrhea. *Gastroenterology* 2009; **136**:1874–86.
- 33 Okeke IN, Nataro JP. Enteroaggregative *Escherichia coli*. *Lancet Infect Dis* 2001; **1**:304–13.
- 34 Ochoa TJ, Salazar-Lindo E, Cleary TG. Management of children with infection-associated persistent diarrhea. *Semin Pediatr Infect Dis* 2004; **15**:229–36.

- 35 Robertson ID, Irwin PJ, Lymbery AJ, Thompson RC. The role of companion animals in the emergence of parasitic zoonoses. *Int J Parasitol* 2000; **30**:1369–77.
- 36 Gilman RH, Marquis GS, Miranda E, Vestegui M, Martinez H. Rapid reinfection by *Giardia lamblia* after treatment in a hyperendemic Third World community. *Lancet* 1988; **1**:343–5.

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