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Prevalence of intestinal parasites among school children in northern districts of West Bank- Palestine

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Summary

OBJECTIVES To assess the prevalence of intestinal parasite infections in northern districts of West Bank, Palestine and to determine associated sociodemographic factors.

METHODS Random sampling of schoolchildren from rural and urban areas was carried out. Participants provided faecal samples and answered a questionnaire about their demographics and hygiene habits. Faecal samples underwent microscopy and PCR to screen for protozoan and helminths.

RESULTS Seven hundred and thirty-five samples were collected from children aged 9.5 years on average. The overall prevalence of parasitic infection was 22.2%. The rates of infections with amoeba, *Giardia intestinalis*, *Entrobius vermicularis* and *Ascaris lumbricoides* were 9.7%, 4.1%, 1.6% and 3.8%, respectively. Real-time PCR was performed to differentiate between *Entamoeba histolytica* and *Entamoeba dispar*. Results showed that 14% of samples positive with microscopy for amoeba were positive for *E. histolytica*. There was no significant association between sex and rates of infections (P-value > 0.05). There were, however, significant association between parasite infections and parents' education, place of residence, washing hands habits (P-value > 0.05). No significant association was found with number of family members or eating in school canteens (P-value > 0.05).

CONCLUSIONS Intestinal parasite infections are endemic in West Bank. Interventions such as health education and sanitation are needed.

keywords intestinal parasitic infections, *Entamoeba histolytica*, *Giardia*, school children, handwashing, Palestine

Introduction

Infections by intestinal parasites are a major public health problem worldwide, especially among children. WHO (2001) estimated that 3.5 billion people are infected by intestinal parasites and 350 million ill by them. *Entamoeba histolytica* infects 500 million people per year, causes disease in 50 million and causes 100 000 deaths annually (WHO 1997a). Most morbidity and mortality caused by amoebiasis occur in developing regions (Walsh 1986). For example, in Bangladesh, it is estimated that approximately 50% of children have evidence of exposure to *E. histolytica* by 5 years of age (Haque *et al.* 1999). *Giardia intestinalis*, a frequent cause of diarrhoea that can have great impact on growth and development of children, affects approximately 200 million people worldwide (WHO 1992). *Ascaris lumbricoides* and *Entrobius vermicularis* are two of the most prevalent intestinal helminths in school-age children (Bethony *et al.* 2006). Worldwide, 320 million school-age children are infected with *A. lumbricoides* (WHO 2007). Infection with *E. vermicularis* is common among primary school children because they are regularly exposed to overcrowded conditions and inadequate sanitation, both associated with infection with this parasite.

Although there have been a few reports of infection rates of intestinal parasites in some local areas of Palestine (Ali-Shtayeh *et al.* 1989; Abu Mourad 2004; Abu Elamreen *et al.* 2007; Hussein *et al.* 2009), no extensive epidemiological survey in the area has been conducted. Protozoan parasitosis is routinely diagnosed by microscopic examination of fresh or fixed stool samples, which is rarely accurate and not strain-specific. This epidemiological survey was performed to profile intestinal parasite infections and identify associated sociodemographic and environmental factors among Palestinian schoolchildren.

Materials and methods

This cross-sectional study was carried out in Nablus, Tulkarm and Qaliqilia districts of North West Bank (Figure 1). The total number of inhabitants of the three districts is 603 355 (Palestinian Central Bureau of Statistics 2009). Participants were selected from schools in urban

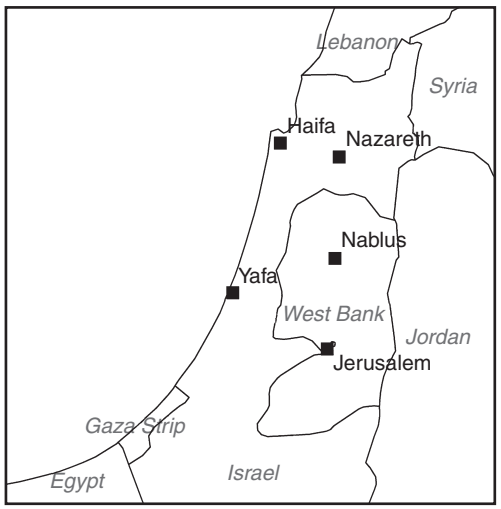

Figure 1 The regional setting of the West Bank and the location of Nablus.

and rural areas. Rural areas including refugee camps comprise 40.6% of the total population of the specified districts.

Stool samples were collected from participants. To detect *E. vermicularis* infection, adhesive cellophane perianal swabs were used to collect specimens. Stool samples were stored in 10% formalin (for stool microscopy) or 100% ethanol (for DNA extraction) until use. Adhesive cellophane perianal swabs were analysed by microscopy at low (100×) magnification for ova of *E. vermicularis* on the day of collection.

In addition, specially designed questionnaires were used to elicit age, gender, place of residence, educational background of the parents, number of family members, number of children per family and hand washing habits.

Stool microscopy

Stool samples were concentrated using formyl-ether concentration technique. Protozoan parasites were examined by staining with Lugol's iodine solution (Moody 1996) using light microscopy at high (400×) magnification. Ova of helminth parasites were examined by direct wet-mount observation at low (100×) magnification. The presence of amoeba and *Giardia* in faecal samples was confirmed by polymerase chain reaction as described below.

DNA extraction

Genomic DNA extraction was performed from approximately 150 mg of faecal samples that were microscopically positive for amoeba and *Giardia* as described above using the QIAamp DNA stool mini test kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions.

Polymerase chain reaction amplification and detection

For amoeba, real-time PCR was performed for the detection and differentiation of *E. histolytica* and *Entamoeba dispar* using the LightCycler (Multiplex Quantitative PCR System, STRATAGENE). Reagents for real-time PCR were purchased from Roche Diagnostics (Manheim, Germany). We used oligonucleotide primers and probes (Sigma Alldrich, Germany) designed for rDNA of *E. histolytica* and *E. dispar* (Blessmann *et al.* 2002) (Table 1). PCRs were in 10 μl containing 1 μl of 1× PCR buffer, 1.2 μl of 25 mM $MgCl_2$, 1 μl of 5 mM dNTP, 1 μl of Taq polymerase (1 U) (PeQlab, Fareham, UK), 1 μl each of sense and anti-sense primer (10 pmole/μl), 0.5 μl each of LC-Red 640- and fluorescin labelled probe (4 pmole/μl), 1 μl of DNA extract (200 ng) and 2.8 μl H_2O. The PCR conditions consisted of 94 °C for 5 min followed by 45 cycles of the following programme: 94 °C for 30 s, 58 °C for 20 s, 72 °C for 20 s. The result was considered positive when a crossing point in the quantification analysis screen was observed.

For *G. intestinalis*, PCR targeting the 18S rRNA gene locus using primers and reaction conditions according to Monis *et al.* (1999) was performed. Primers sequences are listed in Table 1. PCRs were in 50 μl containing 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.25 mM of each dNTP, one unit Taq polymerase (PeQlab, Fareham, UK), 150 nM of each primer and 200 ng DNA extract. The PCR conditions consisted of 94 °C for 5 min followed by 30 cycles of the following programme: 94 °C for 30 s, 58 °C for 30 s, 72 °C for 50 s and an extension reaction at 72 °C for 5 min. PCR products were subjected to electrophoresis in 1.5% (w/v) agarose-TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.3) gels stained with ethidium bromide.

The study was approved by the Medical Ethical Committee at An-Najah National University. Consent forms were signed by the parents of participating schoolchildren. Data were analysed using Statistical Package for Social Sciences (SPSS), Version 16.0 (Chicago, IL, USA).

Results

Study population

Of 808 randomly selected participants, 73 (10%) particularly from rural areas were excluded because of various reasons. Some students failed to give stool

Table 1 Primers and probes used in PCR assays

Target organism	Oligonucleotide	Sequence (5′–3′) and label	References
Entamoeba histolytica	Sense primer	GTA CAA AAT GGC CAA TTC CTT AA CG	Blessmann *et al.* (2002)
	Anti-sense primer	GAA TTG ATT TTA CTC AAC TCT AGA G	
	Probe 1	LC-Red 640-AAC CCC AAT TCC TCG TTA TCC p	
	Probe 2	Fluorescein-GCC ATC TGT AAA GCT CCC TCT CCG A X	
Entamoeba dispar	Sense primer	GTG CAA AGT GGC CAA TTT ATG TAA GCA	Blessmann *et al.* (2002)
	Anti-sense primer	GAA TTG ATT TTA CTC AAC TCT AGA G	
	Probe 1	LC-Red 640-AAC CCC AAT TCC TCG TTA TCC p	
	Probe 2	Fluorescein-GCC ATC TGT AAA GCT CCC TCT CCG A X	
Giardia intestinalis	Sense primer	TCC GGT YGA ATT CTG CC	Monis *et al.* (1999)
	Anti-sense primer	CTG GAA TTA CCG CGG CTG CT	

Table 2 Parasite prevalence by gender of 735 school children in Northern Districts of West Bank

		Entamoeba histolytica/ diaspar		*G. lambilia*		*Entrobius vermicularis*		*Ascaris lumbricoides*		*E. vermicularis/ A. lumbricoides*	
Variable	No.	No.	%	No.	%	No.	%	No.	%	No.	%
Sex											
Boys	423	40	9.4	20	4.7	18	4.2	6	1.4	6	1.4
Girls	312	31	9.9	10	3.2	9	2.9	6	1.9	3	1.0
P-value*		0.900		0.349		0.428		0.770		0.740	
OR (95% CI)		1.00 (0.96, 1.05)		0.98 (0.96, 1.01)		0.98 (0.96, 1.01)		1.00 (0.98, 1.02)		0.99 (0.98, 1.01)	
Total	735	71	9.7	30	4.1	27	3.8	12	1.6	9	1.2

Pearson's chi-square test.

samples and others failed to complete the questionnaire. Thus, 735 faecal samples collected during the study period from 1 February 2008 to 31 May 2008 from school children with a mean age of 9.5 years (range 7–13 years) were analysed. Four hundred and twenty-three (57.5%) were boys.

Prevalence of helminths and protozoa

Table 2 summarizes the sex distribution of parasite infection among school boys and girls children in northern districts of West Bank, Palestine. The overall prevalence of parasite infection among the participants is 20.2% (149/735). The highest prevalence of infection was caused by amoeba (9.7%; 71/735) followed by *Giardia* (4.1%; 30/735). The prevalence rates of *A. lumbricoides* and *E. vermicularis* among participants were 1.6% and 3.8%, respectively. About 1% of participants had mixed infection with *A. lumbricoides* and *E. vermicularis*. Statistical analysis showed no significant association between sex and rates of parasite infections (*P*-value > 0.05) (Table 2).

Comparison of microscopy with PCR results revealed that 94% (30/32) of samples positive for *G. intestinalis* by microscopy also were positive by PCR. All samples positive by microscopy for amoeba were also positive by PCR. Differentiation into *E. histolytica* and *E. dispar* by PCR revealed 1.4% (3/735) of samples to be positive for *E. histolytica vs.* 8.3% (61/735) positive for *E. dispar*.

Significant associations were found between infections and place of residence as well as parents' education (*P*-value < 0.05) (Table 3). Children living in villages and refugee camps (rural areas) were more susceptible to infection. The data showed also that education of parents helps in preventing transmission of parasite infections as there was a significant association between parents' education and rates of parasite infections (*P*-value < 0.05). Number of family members and number of family members living in one room were not associated with the

prevalence of parasite infections (*P*-value > 0.05) (Table 3).

To explore the source of infection with parasites, participants' hygiene habits were investigated (Table 4). There were significant associations between parasite infection and poor personal hygiene habits, as indicated by washing hands before eating or washing fruits and vegetables (*P*-value < 0.05). There was no significant association between infection and eating in the school canteen (*P*-value > 0.05).

Discussion

This study found that 20.2% of school children in northern districts of West Bank are infected by intestinal parasites, most commonly by amoeba and *G. intestinalis*. This is consistent with other studies conducted in different geographic areas of Palestine (Ali-Shtayeh *et al.* 1989; Al-Agha & Teodorescu 2000; Abu Mourad 2004; Abu Elamreen *et al.* 2007) indicating that intestinal parasite infection is an important public health problem in Palestine. For example, in Gaza Strip (Figure 1), the prevalence of intestinal parasites in schoolchildren is 34–53% (Al-Agha & Teodorescu 2000; Astal 2004); 29.8% of interviewees in Gaza reported intestinal parasites among their household members (Abu Mourad 2004). In the same area, the prevalence of *E. histolytica/E. dispar* was reported by Abu Elamreen *et al.* (2007) to be 15%. In West Bank, the only report on intestinal parasites in Nablus showed that the prevalences of *E. histolytica*, *G. intestinalis* and *A. lumbricoides* are 22.9%, 7.3% and 5.7%, respectively (Ali-Shtayeh *et al.* 1989). Similarly high prevalence rates occur mostly in developing countries, namely India, Bangladesh, Iran, Saudi Arabia, Zambia and Turkey (Kang *et al.* 1998; Haque *et al.* 1999; Al-Shammari *et al.* 2001; Ostan *et al.* 2007; Arani *et al.* 2008; Siwila *et al.* 2010).

The comparison of microscopy with PCR for the detection of gastrointestinal protozoa yielded 30 (4.1%) positive faecal samples by PCR *vs.* 32 (4.4%) positive by microscopy for *G. intestinalis,* indicating the specificity of PCR over conventional methodology. Therefore, the prevalence of giardiasis in this study was considered 4.1 based on PCR results. In the same context, real-time PCR showed that only 14% of positive amoeba by microscopy is *E. histolytica*. The latter result, which is consistent with results from other studies with regard to differentiation between the two organisms (Blessmann *et al.* 2002; Verweij *et al.* 2004), shows the importance of using PCR for amoeba to generate accurate diagnoses and epidemiological data (WHO 1997b; Petri *et al.* 2000).

The data of the study showed no significant difference between sex of children and intestinal parasitic infection, although rates of infection were higher in rural than urban areas. Moreover, the degree of parents' education was significantly associated to rates of infection, as were other factors such as hand washing and washing vegetables and fruit. Therefore, intervention programmes

Table 3 Association between demographic variables and parasite infections among 735 Palestinian school children

Variable	No.	Parasite infection no. (%)	*P*-value*	OR (95% CI)
Residence†				
Urban	393	64 (16.2)	0.003	1.31 (1.11, 1.54)
Rural	342	85 (24.8)		
Father education‡				
High	194	27 (13.9)	0.009	1.11 (1.03, 1.19)
Low	541	122 (22.5)		
Mother education‡				
High	153	19 (12.4)	0.005	1.13 (1.05, 1.22)
Low	582	130 (22.3)		
Brothers and sisters no.				
≤3	253	46 (18.2)	0.291	
>3	482	103 (21.3)		
Brothers and sisters no. in one room				
≤3	495	100 (20.2)	1.000	
>3	240	49 (20.4)		

*Pearson's chi-square test.
†Urban: Children living in cities; Rural: children living in villages and refugee camps.
‡High education: Those parents who have university degrees; Low: those parents who do not have university degrees.

Table 4 Association of parasite infection among 735 Palestinian school children in northern districts of West Bank and their hygiene habits

Variable	No.	Parasite infection no. (%)	*P*-value	OR (95% CI)
Washing hands before eating				
Yes	570	32 (05.6)	<0.001	3.25 (2.55, 4.12)
No	165	117 (70.9)		
Washing hands after eating				
Yes	704	140 (19.9)	0.251	
No	31	9 (29.0)		
Washing vegetables and fruits				
Yes	596	39 (02.6)	<0.001	4.64 (3.33, 6.46)
No	139	110 (79.1)		
Eating from the school canteen				
Yes	653	131 (20.0)	0.664	
No	82	18 (22.0)		

including health education and environmental sanitation are required.

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