Intestinal parasitic infections among children in central Albania

A. SEJDINI[*], R. MAHMUD[*], Y. A. L. LIM[*], M. MAHDY[*], F. SEJDINI[†], V. GJONI[‡], K. XHAFERRAJ[§] and G. KASMI[¶]

[*]*Department of Parasitology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[†]*Bacterological Laboratory, Lagjia 'Kongresi I Elbasanit', Elbasan, Albania*
[‡]*Control of Infectious Diseases Department, Institute of Public Health, Alexander Moisu Street 80, Tirana, Albania*
[§]*Department of Gastroenterology and Hepatology, University Hospital Centre 'Mother Teresa', Dibra Street, Tirana, Albania*
[¶]*Department of Parasitology, University Hospital Centre 'Mother Teresa', Dibra Street, Tirana, Albania*

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Although intestinal parasitic infections (IPI) among children remain a global issue, the current information on such infections in Albanian children is very limited. A cross-sectional study of the IPI in 321 children living in the Albanian counties of Tirana (152) and Elbasan (169) was therefore conducted in 2008, with a pre-tested standard questionnaire employed to gather the relevant personal and clinical data. Using formalin–ether concentration and permanent stains, stool samples were examined microscopically for the ova, cysts and oocysts of any parasites. The overall prevalence of IPI was 19% (61 of 321), with protozoan infections (11.5%) apparently more common than infections with soil-transmitted helminths (STH; 8.1%). *Giardia duodenalis* was the parasite most frequently detected (10.9%), followed by hookworm (5.6%), *Ascaris lumbricoides* (1.9%), *Trichuris trichiura* (0.6%), *Cryptosporidium* (0.3%) and *Entamoeba histolytica/dispar* (0.3%). The results of a univariate analysis indicated that the children from Tirana county were significantly more likely to be found infected with STH compared with the children from Elbasan county (12.5% *v.* 4.1%; $P=0.006$). Children sampled in the community were also more likely to be found STH-positive than the children sampled as they attended hospitals and health clinics (10.5% *v.* 6.0%) but this difference did not reach statistical significance. The children found STH-positive were five times more likely to be suffering from diarrhoea than the other children checked in clinical settings ($P=0.004$) and were also more likely to be suffering from abdominal pain ($P=0.054$) and/or diminished appetite ($P=0.016$).

Intestinal parasitic infections (IPI) are still major public-health problems in many developing and developed countries (Hotez, 2008; Hotez *et al.*, 2009; Harhay *et al.*, 2010). Intestinal helminths, for example, affect more than one-sixth of the world's population (De Silva *et al.*, 2003; Bethony *et al.*, 2006; Harhay *et al.*, 2010), with children and pregnant women at particularly high risk (Quihui *et al.*, 2006; Harhay *et al.*, 2010). Among the protozoan parasites of the human intestine, *Giardia duodenalis* and *Cryptosporidium* species are the most common and have been reported to cause multiple waterborne outbreaks of human illness (Harhay *et al.*, 2010). Although most transmission of these protozoa is anthroponotic, their zoonotic transmission has been widely postulated (Applebee *et al.*, 2005; Bajer *et al.*, 2008). Another protozoan parasite, *Entamoeba histolytica*, causes

Reprint requests to: R. Mahmud.
E-mail: rohela@ummc.edu.my.

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amoebic dysentery and has been considered the second most common cause of parasite-attributable death, after malaria (Stanley, 2001, 2003; Gonzales *et al.*, 2009). Among children, IPI may lead to protein-energy malnutrition, iron-deficiency anaemia and deficits in both mental and physical growth (Sackey *et al.*, 2003; Rodríguez-Morales *et al.*, 2006).

In Europe, high prevalences of IPI have been reported in immigrants (20.8%; Rosso and Miotti, 1991) and institutions for the mentally retarded (55.5%; Giacometti *et al.*, 1997) in Italy, among immigrant children in Spain (48%; Huerga Aramburu and López-Vélez, 2004) and in refugees living in Sweden (17%; Persson and Rombo, 1994). Intestinal helminths were found in 1.34% of the Spanish schoolchildren screened by Jarabo *et al.* (1995) and 18.9% of the Turkish orphans investigated by Ozcelik *et al.* (1995). Schoolchildren in Serbia and Spain also frequently harbour IPI, with reported prevalences of 24%–31% (Nikolić *et al.*, 1995, 1998) and 27%–44.8% (Jarabo *et al.*, 1995; Pérez Armengol *et al.*, 1997), respectively. Giardiasis is commonly considered to be a travel-related disease in Europe (Ekdahl and Andersson, 2005; Espelage *et al.*, 2010). Several outbreaks of diarrhoea caused by *G. duodenalis* have been reported among English travellers to Greek resorts (Hardie *et al.*, 1999) and among children attending kindergarten in Norway (Wahl and Bevanger, 2007). In the spring of 2001, after the war in Kosovo, there was an upsurge in the incidence of *Giardia*-related diarrhoea among the Kosovar children living in Mitrovica (Quamilè *et al.*, 2010).

In Albania, 31.9% of children seeking healthcare (Gjoni, 2003) and 47.8% of children living on the peripheries of some major cities (mostly in communities of immigrants from rural areas; Mitrushi, 2008) were found to harbour IPI. Berrilli *et al.* (2006) described how 22 (44%) of 50 stool samples from Albanian children with acute gastro-enteritis were PCR-positive for *G. duodenalis*, with most of the infections apparently associated with intensive outdoor activities during the summer months. Drinking contaminated water and close contact with animals have been postulated as the two main routes for the transmission of *G. duodenalis* to humans in Albania (Berrilli *et al.*, 2006). Recently, *Cryptosporidium* has been reported as an emerging pathogen in Albania, with Gjoni *et al.* (2008) detecting this parasite in 8.7% of 4225 children seeking healthcare. *Trichuris trichiura*, *Ascaris lumbricoides* and *Oxyuris vermicularis* appear to be the most common intestinal helminths in Albania, Mitrushi (2008) finding at least one of these parasites in 246 (40.7%) of 604 young children (aged 3–6 years) from Albanian slums (even though one in three of the subjects of this study had reportedly taken anti-helminthic medication before the study began).

Little is known about the factors contributing to the high prevalence of IPI in Albania, and the subjects of most previous studies on this topic have been inpatients at the same health facility: the University Hospital Centre 'Mother Teresa', in the capital city of Tirana. The aims of the present study were to determine (in both community and clinical settings) the prevalence and distribution of intestinal parasites among the children living in the Albanian counties of Tirana and Elbasan, and to explore possible risk factors for the IPI that were detected. It was hoped that the data collected would provide a better understanding of the epidemiology of IPI in Albania and help the public-health authorities implement better control strategies.

SUBJECTS AND METHODS

Study Area

This study was carried out in the central Albanian counties of Tirana and Elbasan (see Figure), in September–October 2008. The study counties are currently undergoing considerable development and have

FIG. A sketch map of Albania, showing the county boundaries and the locations of the counties of Tirana and Elbasan and the city of Tirana.

heterogeneous populations, partly because they attract many workers (and their families) from elsewhere in the country.

The county of Tirana has a population of about 800,000, living mostly in urban areas (Anon., 2009, 2010), and includes, and is named after, the Albanian capital city of Tirana. The major local industries attract many workers, both from other counties to Tirana and from rural areas of Tirana county to more urban areas, leading to overcrowded urban areas and peri-urban slums with no sanitation, improper waste management and poor infrastructure (Mitrushi, 2008).

The county of Elbasan has a population of about 350,000 who, despite the steel, cement, chrome, timber and soap industries in the county (Anon., 2009, 2010), mostly live in rural communities. Much of the agricultural land has been severely contaminated by pollution from the local industries.

At the time of the last national census (Anon., 2010), the mean number of members of a household in Elbasan county (4.2) was similar to that in Tirana county (4.0) but Elbasan county had a markedly higher level of unemployment (12.7%) than Tirana county (7.9%).

Subjects

A single faecal sample was collected from each of 321 children aged 6 months–16 years. The subjects were investigated 'in the community' (orphans living with foster families in Tirana county and kindergarten children in Elbasan county) or in 'clinical settings' (children attending laboratory diagnostic centres for routine laboratory tests and children seeking treatment in a local hospital). The parents of each subject aged <7 years were requested to collect a faecal sample from the subject, into a screw-capped container. Older subjects were taught how to collect the faecal samples themselves.

Before any samples were collected, the parents of the children and kindergarten directors were told the aims and methods of the study and asked to give their verbal consent (most parents and directors were willing to give consent verbally but felt uncomfortable giving, and declined to give, written consent). A child was only investigated if the relevant consent had been obtained.

Standard Questionnaire

Trained interviewers collected relevant demographic and clinical data on each subject, in interviews with the subjects' parents/guardians. A pre-tested, standardized questionnaire was used to record each subject's age, gender and county of residence, his or her parents' level of education [elementary education is obligatory in Albania (Anon., 2010)], his or her household's size (i.e. the number of people living in the subject's house), and whether the subject lived in a rural or urban area. The interviewers also recorded whether each subject had (self-reported) diarrhoea — defined as unusually loose stools or frequent (at least three/day) stools (Bhutta *et al.*, 1999) — or any (self-reported) symptoms of gastroenteritis (i.e. vomiting, nausea, abdominal pain and/or fever).

Parasitological Examination

Each stool sample was checked microscopically for parasitic cysts, oocysts and ova, both before any concentration (as a fresh smear stained with iodine) and as a wet mount or stained dry smears after formalin–ether concentration.

Dry, stained smears were checked for protozoan cysts (at $\times 100$, after trichome staining) and *Cryptosporidium* oocysts (at $\times 400$, after Ziehl–Neelsen staining).

Faecal samples were initially examined in the Parasitological Laboratory (of the Institute of Public Health) in Tirana city or the Bacteriological Laboratory in Elbasan city. All were re-examined, for quality control and confirmation of the Albanian results, in the Department of Parasitology at the University of Malaya, in Kuala Lumpur.

The children found infected were given appropriate treatment by their family doctors.

Data Analysis

Data were analysed using version 11.5 of the SPSS for Windows software package (SPSS, Chicago, IL). Associations between proportions were explored in χ^2 tests while potential risk factors for IPI were investigated via univariate analysis. A P-value of <0.05 was considered indicative of a statistically significant difference or association.

Ethical Approval

The study protocol was approved by the Medical Ethics Committee of the University of Malaya Medical Centre (reference 764.22).

RESULTS

The 321 enrolled children (178 girls and 143 boys) had a median age of 5 years, most (207) being 'preschool children' aged <7 years. The overall prevalence of (detected) IPI (see Table 1) was 19.0%, with protozoa (11.5%) appearing more common than soil-transmitted helminths (STH; 8.1%). *Giardia duodenalis* appeared to be the most common parasite (10.9%), followed by hookworm (5.6%), *Ascaris lumbricoides*

TABLE 1. *Prevalences of intestinal parasites among 321 children from central Albania*

Parasite	No. and (%) of children
PROTOZOA	
Giardia duodenalis	35 (10.9)
Entamoeba histolytica/dispar	1 (0.3)
Cryptosporidium	1 (0.3)
SOIL-TRANSMITTED HELMINTHS	
Hookworm	18 (5.6)
Ascaris lumbricoides	6 (1.9)
Trichuris trichiura	2 (0.6)
MIXED INFECTIONS (PROTOZOA PLUS HELMINTH)	
Giardia and *Ascaris*	1 (0.3)
Giardia and hookworm	1 (0.3)

(1.9%), *Trichuris trichiura* (0.6%) and, in equal fifth place, *Cryptosporidium* (0.3%) and *Entamoeba histolytica/dispar* (0.3%). Although, in terms of IPI, girls were more likely to be found positive than boys, preschool children were more likely to be found positive than schoolchildren (i.e. children aged 7–16 years), urban children were more likely to be found positive than rural children, and the subjects from Tirana county were more likely to be found positive than their counterparts from Elbasan county, none of these differences reached statistical significance (Table 2).

TABLE 2. *Potential socio–demographic risk factors for intestinal parasitic infection (IPI) among 321 children from central Albania*

Variable	No. and (%) of children: Examined	Found positive for IPI	*P*
AGE (years)			0.62
<7	207	41 (19.8)	
7–16	114	20 (17.5)	
GENDER			0.96
Male	143	27 (18.9)	
Female	178	34 (19.1)	
HOUSEHOLD SETTING			0.74
Rural	28	5 (17.9)	
Urban	254	52 (20.5)	
COUNTY OF RESIDENCE			0.75
Tirana	152	30 (19.7)	
Elbasan	169	31 (18.3)	

Although the girls were also more likely to be found positive for STH infections than the boys, and the schoolchildren more likely to be found to harbour such infections than the preschool children, these differences were also not statistically significant (Table 3). The children from Tirana county appeared to be at three-fold higher risk of acquiring STH than their counterparts in Elbasan county ($P=0.006$) and the children from households that had fewer than five members appeared to be at five-fold higher risk of acquiring STH than the children from larger households ($P=0.012$; Table 3).

The most common clinical manifestations associated with STH infections among the Albanian children seen in clinical settings were diarrhoea, abdominal pain and diminished appetite (Table 4). The children found infected with STH (see Table 4) were at 5.5-fold higher risk of suffering from diarrhoea ($P=0.004$), and STH infections were also significantly (or almost significantly) associated with diminished appetite ($P=0.016$) and abdominal pain ($P=0.054$).

DISCUSSION

The overall prevalence of IPI detected among the Albanian children of the present study (19%) falls marginally below the range of values (19.7%–47.9%) reported for hospitalized Albanian children between 1997 and 2007 (Gjoni *et al.*, 2008). It is much lower than the 37%–89% reported for schoolchildren in Brazil (Basso *et al.*, 2008) and also lower than the prevalences of IPI detected among schoolchildren in neighbouring Serbia (24.6%; Nikolić *et al.*, 1998) and in Turkey (33.4%; Aksoy *et al.*, 2007), but much higher than the value (1.8%) reported for native Italian children by Giordano *et al.* (2001).

TABLE 3. *Results of univariate analysis showing potential socio–demographic risk factors for infection with soil-transmitted helminths (STH) among 321 children from central Albania*

Variable	No. and (%) of children: Examined	Found positive for STH	Odds ratio and (95% confidence interval)	*P*
AGE (years)			1.6 (0.72–3.64)	0.24
<7	207	14 (6.8)		
7–16	114	12 (10.5)		
GENDER			1.3 (0.60–2.74)	0.51
Male	143	10 (7.0)		
Female	178	16 (9.0)		
HOUSEHOLD SETTING			0.9 (0.87–0.94)	0.097
Rural	28	0 (0.0)		
Urban	254	23 (9.1)		
COUNTY OF RESIDENCE			3.0 (1.30–6.98)	0.006
Tirana	152	19 (12.5)		
Elbasan	169	7 (4.1)		
HOUSEHOLD SIZE			5.0 (1.19–21.48)	0.012
Four or fewer members	131	16 (12.2)		
At least five members	83	2 (2.4)		
SOURCE OF SAMPLE			1.8 (0.82–3.75)	0.14
Healthcare facility	168	10 (6.0)		
Community	153	16 (10.5)		

Giardia duodenalis was the most commonly detected intestinal parasite (10.9%) in the current study, which is in agreement with the results of previous studies in Albania (Gjoni, 2003) and Sicily (Giordano *et al.*, 2001). Given the sampling in the present study (in which the subjects investigated were a mix of apparently healthy children sampled in the community and children seeking healthcare), it is perhaps not surprising that the observed prevalence of giardial infection lay between the 13.3% seen in a large study of hospitalized Albanian children with gastroenteritis (Gjoni, 2003) and the 7.1% reported among kindergarten children in a slum area of Albania (Mitrushi, 2008). The prevalences reported for giardial infection (and other

TABLE 4. *Results of univariate analysis showing the levels of association between three symptoms and infection with soil-transmitted helminths (STH), as seen among 151 children as they attended healthcare facilities in central Albania*

Symptom	No. and (%) of children: Examined	Found positive for STH	Odds ratio and (95% confidence interval)	*P*
DIARRHOEA			5.5 (1.63–18.20)	0.004
Reported	11	3 (27.3)		
Not reported	140	7 (5.0)		
DIMINISHED APPETITE			4.0 (1.18–13.41)	0.016
Reported	41	6 (14.6)		
Not reported	109	4 (3.7)		
ABDOMINAL PAIN			3.3 (0.90–12.45)	0.054
Reported	62	7 (11.3)		
Not reported	89	3 (3.4)		

IPI) in the present study are, however, likely to be underestimates of the true prevalences because time constraints and the generally poor responses of the subjects or their parents meant that only one faecal sample from each subject was collected and examined. It is generally recommended that three consecutive faecal samples be collected and examined to give a reasonably accurate estimate of the prevalence of *G. duodenalis* infection (Danciger and Lopez, 1975). It should be noted that, in the aetiology of diarrhoea caused by parasitic protozoa, *Giardia* is the most common culprit, leading to malabsorption and nutritional deficiencies that inhibit a child's growth and development and may contribute to an unhealthy and unqualified future workforce (Dillingham and Guerrant, 2004; Savioli *et al.*, 2006; Harhay *et al.*, 2010).

In the present study, the most common STH infection was hookworm (5.6%), followed by *A. lumbricoides* (1.9%) and *T. trichiura* (0.6%). This appears to be the first report of hookworm infection in Albania, although, in the 1990s, such infection was detected (at a prevalence of 15%) in Bosnians seeking asylum in Sweden (Benzeguir *et al.*, 1999). Hookworm has a cosmopolitan distribution and a relatively long lifespan (Brooker and Bundy, 2009) making its carriage within asylum seekers and other immigrants quite likely. In central Albania, the local farmer's use of human faeces as fertilizer may well facilitate the transmission of hookworms. Chronic infection with STH in childhood may interfere with the human host's physical and mental development, leading to relatively inactive adolescents with relatively poor attention spans (Hotez, 1989; Hall, 1993; Nokes and Bundy, 1994; Sternberg *et al.*, 1997; Hall *et al.*, 2008).

It is not clear why children in Tirana county appear significantly more likely to harbour IPI than their counterparts in neighbouring Elbasan county but the difference may relate to overcrowding and slums, both of which are more common in Tirana county. There has been a recent trend in Albania for the people in the north and south of the country to move towards the centre (and, particularly to Tirana city), where most of Albanian industry is concentrated. This has led to the illegal building of houses and schools in peri-urban areas, without any proper infrastructure or sanitary systems (Mitrushi, 2008; Anon., 2010), and the resultant slum-like conditions probably favour the transmission of many, if not all, IPI (Harhay *et al.*, 2010).

It is also unclear why, in the present study, children living in smaller households (with fewer than five members) appeared at relatively high risk of IPI. One possible explanation is that children from small households are more likely to attend day-care centres where child-to-child transmission of parasites by the faecal–oral is quite likely to occur. There is a growing trend in Albania, and especially in urban Albania, for parents and children to live at some distance from the relatives (e.g. the children's grandparents and aunts) who, traditionally, would have helped with the care of the children. Working parents living in small households are thus increasingly dependent on day-care centres to look after their children.

The present study showed a significant or almost-significant association between STH infection and diarrhoea, abdominal pain and diminished appetite. In a previous study on IPI in Albania, Gjoni (2003) reported an association between 'intestinal parasitosis' (without any more detail of the type of infection) and both diarrhoea and fever. The long-term complications of STH infection in childhood, related to malabsorption and diminished appetite, may cause chronic weakness and general malaise, which, in turn, may diminish learning and working performance (Harhay *et al.*, 2010).

Further studies with larger groups, involving Albanian children of other counties and communities, should improve our knowledge of the burden of IPI among the young. More needs to be done to determine

which symptoms can be caused by each species of intestinal parasite, the main risk factors for infection with each species, and how co-infection can complicate the clinical picture.

Intestinal parasites clearly need to be considered in the differential diagnosis of Albanian children presenting with gastrointestinal illness (especially diarrhoea, abdominal pain and diminished appetite) and in the routine examination of faecal samples. The regular, scheduled screening of immigrants is highly recommended, as is the implementation of improved wastewater treatment and waste disposal (especially in the peri-urban slums). Primary healthcare providers or educators have to play their role in organizing educational training for mothers, foster parents and the staff of day-care centres and kindergartens, on the importance of IPI and the need for good hygiene as a preventive measure. With such interventions, it should be possible to reduce the transmission of intestinal parasites and, perhaps, entirely eliminate some transmission routes (Pezzani *et al.*, 2009). Molecular studies on Albanian *Giardia*, *Cryptosporidium* and hookworm could help identify the parasites, elucidate the dynamics of their transmission and break the chains of transmission (whether water-related, food-related, anthroponotic or zoonotic) via better control and preventive measures.

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